ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

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COMMENTS FROM THE EDITOR

As usual, the reports received were excellent. However, it has been a long time since I have heard anything at all from a great many of you. Don't forget that preliminary reports of your work could be of considerable interest to many of the readers of the Information Exchange. Appearance of a report in the Info-Exchange does not constitute publication (see statement at bottom of front cover); therefore, there is no need to worry about a conflict with later publication in a scientific journal.

In the present issue we are fortunate in having Dr. Nick Karabatsos' annual report of the Working Catalogue, which provides a current update. Also, the Subcommittee on Inter-Relationships among Catalogued Arboviruses (SIRACA) gives us the latest thinking on the bunyaviruses.

Most of you should already have a copy of the published version of the arbovirus catalogue (International Catalogue of Arboviruses, Including Certain Other Viruses of Vertebrates), edited by Dr. T.O. Berge and printed in 1975. If your copy is lost or worn out, or if one of your senior workers does not have a copy of his/her own, I still have a number of them left and will be glad to send you one, free of charge. However, as with all good things, the supply is limited, so please do not request a copy unless it is truly needed and will be used.

The deadline for reports for Issue No. 37 of the Arthropod-borne Virus Information Exchange is September 1, 1979. Mark your calendars. Reports from outside the United States should be sent air mail to assure prompt arrival. In the interest of economy, it is preferred that reports be typed single space, with double space between paragraphs. The address:

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REPORT OF THE SUBCOMMITTEE ON INTER-RELATIONSHIPS OF CATALOGUED ARBOVIRUSES (SIRACA)

SIRACA met in November, 1978 to review the serologic classification of viruses in the family Bunyaviridae. Viruses were classified step-wise by 1) group, 2) complex, 3) virus (or type), 4) subtype, and 5) variety. Primarily, the decision to place a virus in one of the above sets was based on differences or similarities by the HI and neutralization test. In some cases the CF reaction was also considered, especially for grouping of viruses.

SIRACA reviewed data from published and unpublished sources. Viruses which are not catalogued were also considered where data were available. Ungrouped Bunyaviruses were not considered.

SIRACA invites comments from the scientific community, especially if an investigator disagrees with the placing of a virus. This classification is tentative and will certainly change as new data become available. Please supply data with your comments so that SIRACA can reconsider the classification of any virus deemed incorrectly placed.

The following Table represents SIRACA's current proposed classification. The members of SIRACA are: Robert Shope, chairman; Walter Brandt, Charles Calisher, Jordi Casals, Robert Tesh, and Michael Wiebe.

Group	Complex	Virus	Subtype	Variety
Anopheles A	Anopheles A	Anopheles A CoAr3624		
	Lukuni	Lukuni		
	ColAn57389	ColAn57389		
	Tacaiuma	Tacaiuma CoAr1071	CoAr1071	CoAr1071 CoAr3627
Bunyamwera	Bunyamwera	Bunyamwera Germiston Shokwe Batai Ilesha Birao Tensaw Cache Valley	Cache Valley	Cache Valley Tlacotalpan
			Maguari	Maguari CbaAr426

Group	Complex	Virus	Subtype	Variety
Bunyamwera (continued)				
		Northway		
		Santa Rosa		
		Lokern		
	Wyeomyia	Wyeomyia		
		Taiassui		
		Anhembi		
		Sororoca		
	Main Drain	Main Drain		
	Kairi	Kairi		
Bwamba	Bwamba	Bwamba	Bwamba	
			Pongola	
C	Caraparu	Caraparu	Caraparu (Belem)	
			Caraparu (Trinidad)	
			Ossa	
		Apeu		
	Madrid	Madrid		
	Marituba	Marituba	Marituba	
			Murutucu	
			Restan	
		Nepuyo	Nepuyo	Nepuyo
				63U11
		Gumbo Limbo		
	Oriboca	Oriboca	Oriboca	
			Itaqui	
California	California encephalitis	California encephalitis	California encephalitis	
			LaCrosse	LaCrosse
				snowshoe hare
			Tahyna	Tahyna
				Lumbo
			San Angelo	
			Inkoo	
		Melao	Melao	
			Serra do Navio	
			Keystone	
			Jamestown Canyon	Jamestown Canyon
				South River
		Trivitattus		
	Guaroa	Guaroa		

Group	Complex	Virus	Subtype	Variety
Capim	Capim	Capim		
	Guajara	Guajara	Guajara	Guajara Gu71U350
	Bushbush	Bushbush	Bushbush Benfica Gu71U344	
		Juan Diaz		
	Acara	Acara Moriche		
	Benevides	Benevides		
Guama	Guama	Guama Moju Ananindeua Mahogany Hammock		
	Bimiti	Bimiti		
	Timboteua	Timboteua		
	Catu	Catu		
	Bertioga	Bertioga		
Koongol	Koongol	Koongol Wongal		
Mirim	Mirim	Mirim		
	Minatitlan*	Minatitlan		
Olifantsvlei	Olifantsvlei	Olifantsvlei	Olifantsvlei	Olifantsvlei Bobia
	Botambi	Botambi		

*The relationship of Minatitlan to Mirim is subject to confirmation.

Group	Complex	Virus	Subtype	Variety
Patois	Patois	Patois		
		Shark River		
	Zegla	Zegla		
		Pahayokee		
Simbu	Simbu	Simbu		
	Akabane	Akabane		
		Yaba-7		
	Shamonda	Shamonda		
		Sango		
	Sabo	Sabo		
	Sathuperi	Sathuperi		
	Shuni	Shuni		
		Aino	Aino	Aino
				Kaikalur
	Thimiri	Thimiri		
	Nola	Nola		
	Manzanilla	Manzanilla	Manzanilla	
			Ingwavuma	
			Mermet	
			Inini	
		Buttonwillow		
	Oropouche	Oropouche		
		Utinga		
		Bradypus		
Tete	Tete	Tete	Tete	
			Bahig	
			Matruh	
			Tsuruse	
		Batama		
Anopheles B	Anopheles B	Anopheles B		
		Boraceia		

Group	Complex	Virus	Subtype	Variety
Bakau	Bakau	Bakau		
		Ketapang		
CHF-Congo	no complex assigned	CHF-Congo		
		Hazara		
Kaisodi	Kaisodi	Kaisodi		
		Lanjan		
		Silverwater		
Mapputta	Mapputta	Mapputta		
	Maprik	Maprik		
		Gan Gan		
	Trubanaman	Trubanaman		
Nairobi S.D.	no complex assigned	Nairobi S.D.	Nairobi S.D.	Nairobi S.D.
		Dugbe		Ganjam
Phlebotomus fever				
	Candiru	Candiru		
		Itaituba		
		Nique		
	Punta Toro	Punta Toro		
		Buenaventura		
	Saint-Floris	Saint-Floris		
		Gordil		
	no complex assigned	Aguacate		
		Anhanga		
		Arumowot		
		Bujaru		
		Cacao		
		Caimito		
		Chagres		
		Chilibre		
		Frijoles		
		Icoaraci		
		Itaporanga		
		Karimabad		
		Pacui		

Group	Complex	Virus	Subtype	Variety
Phlebotomus fever (continued)				
		Salehabad		
		SudAn754-61		
		SF-Naples		
		SF-Sicilian		
		Urucuri		
Sakhalin	Sakhalin	Sakhalin	Sakhalin	
			Tillamook	
		Taggert		
		Clo Mor		
		Avalon		
Thogoto	Thogoto	Thogoto	Thogoto	
			SiAr126	
Turlock	Turlock	Turlock	Turlock	
			Umbre	
			M'Poko	
Uukuniemi	Uukuniemi	Uukuniemi	Uukuniemi	
			Oceanside	
		Grand Arbaud		
		Manawa		
		Zaliv Terpeniya		
		Ponteves*		
		EgAn1825-61*		

*Since no neutralization or HI tests are recorded for Ponteves and EgAn1825-61, their placement is tentative.

The AMERICAN COMMITTEE ON ARTHROPOD-BORNE VIRUSES

1978 ANNUAL REPORT ON THE CATALOGUE OF ARTHROPOD-BORNE AND SELECTED VERTEBRATE VIRUSES OF THE WORLD*

by

THE SUBCOMMITTEE ON ARTHROPOD-BORNE VIRUS INFORMATION EXCHANGE

I. Objectives:

The objectives of the Catalogue are to register data concerning occurrence and characteristics of newly recognized arthropod-borne viruses and other viruses of vertebrates of demonstrated or potential zoonotic importance, and to disseminate this information at quarterly intervals to participating scientists in all parts of the world; to collect, reproduce, collate, and distribute current information regarding registered viruses from published materials, laboratory reports, and personal communications; and to prepare and distribute an annual summary of data extracted from catalogued virus registrations.

II. Materials and Methods:

Viruses are registered and information supplied on a voluntary basis, usually by scientists responsible for their isolation and identification. New registration cards, information concerning registered viruses, and pertinent abstracts of published literature are distributed at quarterly intervals to participating laboratories. Abstracts of published articles dealing with catalogued viruses are reproduced by special arrangements with the editors of Biological Abstracts, Abstracts on Hygiene, and the Tropical Diseases Bulletin.

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*The Catalogue is supported by the Center for Disease Control, Atlanta, Georgia.

NOTE: This report is not a publication and should not be used as a reference source in published bibliographies.

Distribution of Catalogue Material: At the start of 1978, 163 mailings of Catalogue material were being made. During the year, 1 participant was dropped and 3 new participants were added to the mailing list. At the end of the year, 165 mailings of Catalogue material were being made, including 59 within the U.S.A. and 103 to foreign addresses. Distribution by continent was: Africa 15, Asia 21, Australasia 7, Europe 35, North America 71, and South America 16.

Abstracts and Current Information: A total of 580 abstracts or references were coded by subject matter and distributed to participants during 1978. Of this total, 448 were obtained from Biological Abstracts, 117 from Abstracts on Hygiene and the Tropical Diseases Bulletin, and 15 from current journals, personal communications, or other sources. A total of 11,412 references or units of information have been issued since the start of the program.

Registration of New Viruses: Twenty new viruses were registered during 1978. As of December 1977, the Catalogue contained 388 registered viruses. With the acceptance of twenty new virus registrations during 1978, the total number of registered viruses increased to 408 as of December 1978. The viruses registered during 1978 are listed below:

Virus Name	Recommended Abbreviation	Country	Source	Antigenic Group
New Minto	NM	USA	Ixodid ticks	SAW
Santa Rosa	SAR	Mexico	Mosquitoes	BUN
Chim	CHIM	USSR	Ixodid ticks	
Kyzylagach	KYZ	USSR	Mosquitoes	A
Araguari	ARA	Brazil	Opposum	
Inhangapi	INH	Brazil	Phlebotamine flies	
Itaituba	ITA	Brazil	Opposum	PHL
Mosqueiro	MQO	Brazil	Mosquitoes	HP
Serra do Navio	SDN	Brazil	Mosquitoes	CAL
Fort Morgan	FM	USA	Bedbugs	A
Telok Forest	TF	Malaysia	Monkey	TR
Slovakia	SLO	Czechoslovakia	Argasid ticks	
Batama	BMA	Cent. Afr. Emp.	Bird	TETE
Bobia	BIA	Cent. Afr. Emp.	Mosquitoes	OLI
Cabassou	CAB	French Guiana	Mosquitoes	A
Inini	INI	French Guiana	Bird	SIM
Rochambeau	RBU	French Guiana	Mosquitoes	
Sakpa	SPA	Cent. Afr. Emp.	Rodent	
Tonate	TON	French Guiana	Bird	A
Buenaventura	BUE	Colombia	Phlebotamine flies	PHL

These recently registered viruses were isolated between 1964 and 1976. One virus was isolated in 1964 (BUE), one in 1966 (SDN), one in 1968 (CAB), four in 1969 (ARA, BIA, INH, KYZ), two in 1970 (BMA, MQO), one in 1971 (CHIM), four in 1972 (ITA, SAR, SPA, TF), five in 1973 (FM, INI, NM, RBU, TON), and one in 1976 (SLO).

One (FM) of the above viruses was evaluated as an Arbovirus by the SEAS* Subcommittee. Two additional viruses (CAB, TON) were evaluated as Probable Arboviruses, while another (SLO) was considered to be Not Arbovirus. All the others were evaluated as Possible Arboviruses.

Only Tonate virus has been isolated from man, and has been reported to produce a benign febrile disease in man.

Reissue of Previous Virus Registrations: Updated registrations were prepared on the newer registration forms for Powassan, Silverwater, and Snowshoe hare viruses. The updated registrations replaced the previous registration cards for these three viruses.

Antigenic Grouping: In the past year, the SIRACA† Subcommittee has reviewed the antigenic relationships of viruses belonging to serogroups of the Bunyamwera Supergroup (See SIRACA Subcommittee Annual Report in this issue of Infoexchange Newsletter). Changes in antigenic classification arising from these reviews should be considered tentative and are subject to further change with the introduction of additional or new data. Several new serogroups have been formed both as a result of virus registration activity this past year and as a result of the SIRACA Subcommittee reviews. The Hart Park serogroup has been expanded to five members by incorporation of the former Mossuril serogroup (Mossuril, Kamese) and by inclusion of the newly registered Mosqueiro virus. Registration data presented for Mosqueiro virus indicates that this virus is antigenically related by CF to both Hart Park and Kamese viruses. The Sawgrass serogroup consists of the recently registered New Minto virus and Sawgrass virus, both of which have been demonstrated to be rhabdoviruses. The Tanjong Rabok antigenic group contains Tanjong Rabok virus and the newly registered Telok Forest virus. Both viruses were isolated in Malaysia. The SIRACA Subcommittee has determined that Mirim and Minatitlan viruses show a sufficiently close antigenic relationship and that these two viruses should constitute the Mirim antigenic group. As a consequence, both viruses have been removed from the collection of viruses placed in the Bunyamwera Supergroup but not assigned to any serogroup (SBU). A recent publication describes the isolation of another Australian virus that is antigenically related to Wallal virus (1). Since only Wallal virus is registered at present, the Wallal serogroup consists of a single registered virus.

Changes in antigenic classification resulting from the review by SIRACA will be discussed in those sections giving specific information for each of the antigenic groups.

* Subcommittee on Evaluation of Arthropod-Borne Status. T.H.G. Aitken (Chairman), R.W. Chamberlain, D.B. Francy, J.L. Hardy, D.M. McLean, A. Rudnick, and J.P. Woodall.

† Subcommittee on Interrelationships Among Catalogued Arboviruses. R.E. Shope (Chairman), W.E. Brandt, C.H. Calisher, J. Casals, R.B. Tesh, and M. Wiebe.

Taxonomic Status of Registered Viruses: In almost all instances, changes in the taxonomic status of a registered virus have resulted from observations obtained by electron microscopy. Thus, New Minto virus and its antigenic relative, Sawgrass virus, have been observed to be "bullet-shaped" particles or rhabdoviruses (see individual registration cards for recent information). Khasan, Razdan, and Tamdy viruses, serologically ungrouped agents isolated in the USSR, provisionally have been designated as bunyavirus-like viruses on the basis of their morphology and size characteristics (2-4). Physicochemical and electron microscopic characteristics suggest that Wallal virus is an orbivirus (1). Electron microscopic examination of Belmont virus (5), a serologically ungrouped virus from Australia, and of Avalon and Sakhalin viruses (4), Sakhalin serogroup members, suggest that they possess bunyavirus-like properties.

Synopsis of Information in Catalogue: This synopsis has been compiled primarily to provide a short review of the viruses included in the Catalogue. The following tabulations are designed to draw together groups of viruses showing certain characteristics in common, listing viruses according to their demonstrated serological relationships and known taxonomic status and, where appropriate, by principal arthropod vector. Isolations from arthropod and animal hosts, continental distribution, involvement in human disease, and arbovirus status are indicated. Other tables summarize numbers of viruses assigned to presently recognized antigenic groups; chronology and areas of isolations of registered viruses; continental distribution by groups; numbers of viruses recovered from naturally infected arthropods and vertebrates; association with human disease; and evaluation of arthropod-borne status of members in various serogroups.

Table 1. Alphabetical listing of registered viruses: Table 1 presents an alphabetical listing of the 408 viruses registered in the Catalogue as of December 1978. Also, a recommended abbreviation is given for each virus, which has been formulated according to the guidelines established by the American Committee on Arthropod-Borne Viruses (6). All too often, abbreviations are employed in publications which are of the author's choosing and which do not conform to the recommended abbreviation. Their use is confusing, contrary to established guidelines, and erodes a portion of the effort of the Arbovirus Information Exchange program. All arbovirologists who plan to employ abbreviations in print should make every effort to use the recommended abbreviations.

Antigenic groups to which viruses have been assigned also are shown in this table. If no antigenic group is given, the virus is ungrouped and indicates that it has not been demonstrated to be serologically related to any other known arbovirus.

Table 2. Antigenic groups of registered viruses: The originally described antigenic groups of arboviruses were designated by letters A, B, and C; but in present practice, the first discovered virus of a newly recognized serogroup lends its name to the antigenic cluster. Before a virus can be assigned to an antigenic group, it must be shown to be serologically related to, but clearly distinguishable from a previously isolated virus.

The listing in Table 2 shows that 54 antigenic groups have been established for viruses registered in the Catalogue. There are several instances in which only a single virus is shown in an antigenic group. That is so because one or more antigenic relatives of that virus have not been registered.

It is also noted that the Bunyamwera Supergroup consists of 12 distinct antigenic groups as well as a collection of viruses (Bunyamwera Supergroup Unassigned) which antigenically fall into the Supergroup but which lack a close antigenic relationship to any other virus in the Supergroup. The Bunyamwera Supergroup was formulated to reflect low level but reproducible intergroup relationships usually by complement-fixation and/or hemagglutination-inhibition reactions. Registered viruses belonging in the Bunyamwera Supergroup constitute approximately one-fourth of all registered viruses. It has been demonstrated that all Supergroup viruses examined possess similar, if not identical, morphologic and morphogenetic characteristics (7,8) as well as other biochemical properties. In accordance with the present international taxonomic scheme, Supergroup viruses have been designated to form the Bunyavirus genus within the family Bunyaviridae by the International Committee on Taxonomy of Viruses (ICTV) (8,9).

Table 3. Initial isolations by decade and country of origin: Table 3 lists the initial isolation of specific registered viruses by the decade of discovery and according to the continent or subcontinent and country in which each was first discovered. Because of the large number of virus names involved, abbreviations are employed. These abbreviations and the associated complete names of the respective viruses may be found in Table 1.

Table 4. Initial isolation of viruses by continent, country, and chronological period: Similar data were utilized in Tables 3 and 4, though they were subjected to slightly different analyses and were presented in a different format. Periods or locations which show high numbers of virus isolations undoubtedly reflect the net effect of a number of contributing factors such as the change in emphasis of field programs from a search for viruses causing specific diseases to a systematic search for viruses, new or known, in their natural ecological niche in a given geographical area, refinements in isolation and identification techniques, improved communication between arbovirus laboratories, and more rapid dissemination of new information, as well as the presence in a given area of an arbovirus laboratory with highly active and effective field programs.

Tables 5 through 27 list registered viruses by serogroup with information regarding isolations from arthropod vectors and vertebrates, and geographic (by continent) distribution based on virus isolation. Data also are presented regarding production of disease in man in nature or by laboratory infection, evaluation of arbovirus status, and proved or provisional taxonomic status. Where possible, sets of viruses were grouped additionally according to their actual or suspected principal arthropod vector and by taxonomic status.

The data presented in these tables clearly illustrate the salient features characteristic of each set or subset of viruses. Thus, the reader is urged

to carefully examine the tables for information that may be of specific interest or that will provide an overview of the general characteristics of a given group of viruses.

Table 5. Group A arboviruses: Alphaviruses are clearly mosquito associated, although a few have been isolated from other arthropods. They are also associated with birds or small mammals.

With the addition of four recently registered viruses this past year, this serogroup now consists of 24 members. Two of the additions, Cabassou and Tonate viruses, were isolated in French Guiana, and both were found to be members of the VEE complex. Tonate has been isolated twice from the blood of human beings and it has been reported to produce a benign febrile illness in man. A third addition, Kyzylagach virus, is antigenically very closely related to Sindbis virus, and it was isolated in the USSR from mosquitoes. The final new member, Fort Morgan virus, was isolated in the USA from birds and swallow bedbugs, and it is most closely related to WEE virus.

Two isolations of Ndumu virus have been recovered from the organs of cattle in Rhodesia. These represent the first isolations of Ndumu virus from naturally infected vertebrates.

Semliki Forest virus was associated with a fatal laboratory-acquired infection in man, and it was isolated from CSF and brain. This is not the first involvement of this virus in a laboratory-acquired infection, although the previous episode was a subclinical infection.

One-half of the registered alphaviruses have been implicated in causing infections in man, either in nature or in the laboratory.

Tables 6, 7, and 8. Group B viruses. Of the 60 registered flaviviruses, 47% have been placed in the mosquito-associated category (Table 6), 25% are considered to be tick-borne (Table 7), and 28% are categorized as not being associated with a proven arthropod vector (Table 8).

Twenty-three of the 28 registered group B viruses which are mosquito-associated (Table 6) are rated as Probable Arbovirus or Arbovirus. The group B tick-borne viruses (Table 7) contain four registered viruses, Absettarov, Hanzalova, Hypr, and Kumlinge, which are very closely related or indistinguishable by conventional serological techniques, though they are said to be clearly differentiated on the basis of clinical, epidemiological, and ecological markers from RSSE and other members of the same complex.

With the exception of two members, none of the rest of the registered flaviviruses placed in the "no arthropod vector demonstrated" category (Table 8) are rated above Possible Arbovirus by SEAS. Seven members are rated as Probably not or Not Arbovirus.

Tables 9, 10, 11, 12, 13, and 14. Bunyamwera Supergroup: Recently, SIRACA has re-evaluated antigenic relationships of viruses in the Bunyamwera Supergroup and decided that Mirim and Minatitlan viruses should constitute the Mirim serogroup on the basis of their antigenic relationship to each other. Both viruses were formerly placed in the Bunyamwera Supergroup, unassigned. There are now 12 antigenic sets of viruses plus the unassigned viruses that comprise the Supergroup.

Table 9. Bunyamwera group: The recently registered Santa Rosa virus, isolated from mosquitoes collected in Mexico, is a new member of the Bunyamwera serogroup.

SIRACA considers Calovo and Batai viruses to be serologically indistinguishable. Both of these viruses were upgraded by SEAS from Possible Arbovirus to Probable Arbovirus.

Following recent re-evaluations, SIRACA has concluded that four complexes comprise the Bunyamwera serogroup. Two of the complexes consist of a single virus each. The subsets are given as follows:

1. Bunyamwera [Bunyamwera, Germiston, (Shokwe)*, Batai, Ilesha, Birao, Tensaw, Cache Valley, Northway, Santa Rosa, and Lokern].

2. Wyeomyia [Wyeomyia, (Taiassui), Anhembi, and Sororoca].

3. Main Drain [Main Drain].

4. Kairi [Kairi].

In the Bunyamwera complex, Cache Valley virus consists of subtypes Cache Valley and Maguari. Further, Tlacotalpan is considered to be a variety of the Cache Valley subtype while (CbaAr 426) is considered to be a variety of the Maguari subtype.

Provisionally, SIRACA has removed Guaroa from the Bunyamwera group and placed it in the California group on the basis of its HI and NT relationships to members of the California group.

Table 10. Bwamba and Group C viruses: SIRACA has determined that the Bwamba serogroup consists of the Bwamba complex containing Bwamba virus with subtypes Bwamba and Pongola.

Recently, an additional isolate of Bwamba virus has been obtained from the blood of a febrile child.

The group C viruses have been closely associated with mosquito vectors and small animals, particularly rodents. Only two of these viruses have not been isolated from man.

* ( ): Presently unregistered.

SIRACA believes that this group forms 4 complexes as follows:

1. Caraparu [Caraparu, Apeu]. Caraparu virus consists of subtypes Caraparu-Belem, Caraparu-Trinidad, and Ossa virus.

2. Madrid [Madrid].

3. Marituba [Marituba, Nepuyo, Gumbo Limbo]. Subtypes of Marituba virus are Marituba, Murutuca, and Restan viruses. (63 U 11)* is considered to be a variety of Nepuyo virus.

4. Oriboca [Oriboca]. Oriboca and Itaqui viruses are considered to be subtypes.

Table 11. California and Capim group viruses: The placement of Guaroa virus in the California group and the registration of Serra do Navio virus increases the number of viruses in the group to 13. Serra do Navio virus was isolated from mosquitoes collected in Brazil.

All the California group viruses are associated with mosquito vectors and four members have been recovered from naturally infected rodents. La Crosse and Tahyna viruses have been isolated from man. Three isolations of Tahyna virus have been recovered from the blood of man in Tajik, SSR. On the basis of virus isolation, the geographic distribution of Tahyna now includes Asia as well as Africa and Europe.

SIRACA has decided that two complexes constitute the CAL serogroup. The first complex contains three viruses and the second contains a single virus as follows:

1. California encephalitis [California encephalitis, Melao, Trivittatus]. California encephalitis virus subtypes: California encephalitis, La Crosse, Tahyna, San Angelo, Inkoo. La Crosse virus varieties: La Crosse, Snowshoe hare. Tahyna virus varieties: Tahyna, Lumbo. The Lumbo registration was withdrawn previously because it was considered to be a strain of Tahyna. SIRACA's designation of Lumbo virus as a variety of Tahyna virus acknowledges that there are probably slight differences between the two viruses. Melao virus subtypes: Melao, Serra do Navio, Keystone, Jamestown Canyon. Jamestown Canyon virus varieties: Jamestown Canyon, (South River)*.

2. Guaroa [Guaroa]. Because of HI and NT relationships of Guaroa virus to members of the CAL group, SIRACA placed it in this group.

The Capim group viruses are associated with mosquito vectors, and three of the members have been isolated from rodents. None of these six viruses have been implicated in causing disease in man.

* ( ): Presently unregistered.

In the judgment of SIRACA, this serogroup consists of four complexes:

1. Capim [Capim, Guajara]. Guajara virus varieties: Guajara, (GU 71 u350)*.

2. Bushbush [Bushbush, Juan Diaz]. Bushbush virus subtypes: Bushbush, (BeAn 84381), (GU 71 u344)*.

3. Acara [Acara, Moriche].

4. (BeAn 153564) [BeAn 153564].

Table 12. Guama, Koongol, Olifantsvlei, and Patois group viruses: Guama group viruses have been found only in the western hemisphere. Catu and Guama viruses have been isolated from man and have been implicated in infections of man acquired in nature.

In the judgment of SIRACA, the Guama serogroup consists of five complexes:

1. Guama [Guama, Moju, (Ananindeua)*, Mahogany Hammock].

2. Bimiti [Bimiti].

3. (Timboteaua) [(Timboteaua)*].

4. Catu [Catu].

5. Bertioga [Bertioga].

Both Koongol group viruses were isolated in Australia and very little is known about them.

The Olifantsvlei group now consists of three members, and all three were isolated in Africa. Bobia virus was registered within this past year and Botambi virus was placed within this serogroup on the basis of serological studies conducted at the Institute Pasteur Dakar.

SIRACA has decided that the OLI serogroup consists of two complexes:

1. Olifantsvlei [Olifantsvlei]. Olifantsvlei virus varieties: Olifantsvlei, Bobia.

2. Botambi [Botambi].

Viruses of the Patois group have been isolated only in North America, and they appear to be associated with mosquito vectors and rodent hosts.

SIRACA suggests that this serogroup contains two complexes, each with two virus members:

* ( ): Presently unregistered.

1. Patois [Patois, Shark River].

2. Zegla [Zegla, Pahayokee].

Table 13. Simbu group viruses: The recently registered Inini virus was isolated from a bird in French Guiana.

A number of the Simbu group viruses have been isolated from Culicoides as well as mosquitoes. None have been recovered from rodents. Most have been isolated from birds or livestock. Oropouche and Shuni viruses are the only members that have been isolated from man. Oropouche virus has caused frequent large outbreaks of disease among the human population in Brazil.

SIRACA has allocated the Simbu group viruses to 10 complexes, with 5 complexes containing only a single virus.

1. Akabane [Akabane, (Yaba-7)*].
2. Shamonda [Shamonda, Sango].
3. Sabo [Sabo].
4. Sathuperi [Sathuperi].
5. Shuni [Shuni, Aino].
6. Simbu [Simbu].
7. Thimiri [Thimiri].
8. Nola [Nola].
9. Manzanilla [Manzanilla, Buttonwillow]. Manzanilla virus subtypes: Manzanilla, Ingwavuma, Mermet, Inini.
10. Oropouche [Oropouche, (Utinga)*, (Bradypus)*].

Table 14. Tete group and unassigned (SBU) viruses: All the Tete group viruses have been recovered from birds, including the newly registered Batama virus isolated in Africa. Only two of the members (Bahig, Matruh) have been recovered from any kind of a vector, namely ixodid ticks.

SIRACA has assigned the members to a single complex consisting of 2 viruses, with 1 of the viruses having 3 subtypes.

1. Tete [Tete, Batama]. Tete virus subtypes: Tete, Bahig, Matruh, Tsuruse.

The unassigned viruses have been decreased by three. Botambi virus has been added to the Olifantsvlei group, and Mirim and Minatitlan have formed the Mirim serogroup.

* ( ): Presently unregistered.

Of the remaining unassigned members, three (Bamboa, Guaratuba, and Jurona) have been recovered from mosquitoes, while the last, Kaeng Khoi virus, has been isolated from cimicid bugs.

Table 15. Phlebotomus fever group viruses: Thus far, intergroup antigenic relationships have not been demonstrated between the PHL group and members of the Bunyamwera Supergroup. However, representative members of the PHL group have been examined by electron microscopy and they have been found to be identical in morphology and morphogenesis to Bunyamwera virus. They have been designated as bunyavirus-like though their precise taxonomic status is officially unresolved at present.

The registration of Buenaventura virus, from phlebotamine flies collected in Colombia, and Itaituba virus, from a marsupial trapped in Brazil, increases the number of registered PHL group viruses to 24.

The majority of the group members are associated with phlebotamine flies, while 5 of these viruses have been isolated from man and have been implicated in the production of disease in man.

SIRACA has designated three complexes for this group, although the majority of the members are listed as separate distinct viruses without being assigned to a complex.

1. Candiru [Candiru, Nique]. Candiru virus subtypes: Candiru, Itaituba.

2. Punta Toro [Punta Toro, Buenaventura].

3. Saint-Floris [Saint-Floris, Gordil].

Separate viruses not assigned to a complex: SF-Naples; SF-Sicilian with subtypes SF-Sicilian, (ISS PHL-3)*; Caimito; Anhanga; Bujaru; Cacao; Frijoles; Aguacate; Chagres; Icoaraci; Itaporanga; (SudAn 754-61)*; Karimabad; Salehabad; Pacui; Arumowot; Chilibre.

Table 16. Tick-borne groups other than group B viruses: Members of these five minor antigenic groups also have been characterized as being bunyavirus-like.

A low-titered relationship by CF, fluorescent antibody, and indirect hemagglutination has been demonstrated between Congo and NSD viruses. SIRACA has decided that these relationships are no greater than those used to establish the Bunyamwera Supergroup and that the Congo and NSD serogroups should be kept as distinct sets.

SIRACA has designated CHF-Congo and Hazara viruses as separate viruses without assigning them to a complex.

Members of the Kaisodi group have not been involved in causing infections of man. A single complex has been assigned to this serogroup, and it contains three viruses.

* ( ): Presently unregistered.

1. Kaisodi [Kaisodi, Lanjan, Silverwater].

Nairobi sheep disease virus is an important cause of veterinary disease, while both Dugbe and Ganjam viruses have been isolated repeatedly from ticks taken off domestic animals. Both Dugbe and Ganjam viruses have caused a febrile illness in man. In the case of NSD, one infection in man resulted in a febrile illness, while three others resulted in subclinical serologic conversions.

Dugbe and NSD viruses are designated as separate viruses without being assigned to a complex. Pending further clarification of antigenic relationships, SIRACA considers Ganjam to be a variety of NSD.

Members of the Sakhalin group provisionally have been designated bunyavirus-like on the basis of electron microscopic observations of Avalon and Sakhalin viruses.

SIRACA has indicated that the Sakhalin group consists of a single complex containing four virus types. The viruses are Sakhalin, Taggert, Clo Mor, and Avalon. Subtypes of Sakhalin virus are Sakhalin and (Tillamook)*.

Thogoto virus has been isolated from man and has been involved in the production of disease in man.

SIRACA considers (SiAr 126)* virus, an antigenic relative of Thogoto virus, to be a subtype.

Hemagglutination-inhibition antibodies to Uukuniemi virus have been detected in the sera of human beings residing in Europe.

Members of this serogroup have been assigned to a single complex.

1. Uukuniemi [Uukuniemi, Grand Arbaud, Manawa, Zaliv Terpeniya, Pontevest, (EgAr 1825-61)*†]. Uukuniemi virus subtypes: Uukuniemi, (Oceanside)*.

Table 17. Tick-borne groups other than group B viruses: While the viruses in Table 17 also are tick-borne agents, they differ taxonomically from those in Table 16 in that they have been classified as orbiviruses in the family Reoviridae. The oribiviruses are relatively resistant to lipid solvents, are inactivated at acid pH, and possess a double stranded RNA genome.

Only Colorado tick fever virus of the CTF group and Kemerovo virus of the KEM group have produced disease in man.

Table 18. Tick-borne groups other than group B viruses: Members of these five minor antigenic groups have not been officially classified taxonomically.

* ( ): Presently unregistered.

† Since no NT or HI tests are recorded for Ponteves and (EgAr 1825-61)*, their placement is tentative and speculative.

Only Quaranfil virus of the QRF group has been implicated in causing disease in man in nature.

Tables 19, 20, 21, and 22. Minor antigenic groups of viruses: All the viruses listed in these tables are members of minor antigenic groups. Viruses of the serogroups listed in Table 19 are characterized taxonomically as bunyavirus-like. All virus members of these minor antigenic serogroups have been primarily associated with mosquito vectors.

SIRACA has designated four complexes for viruses of the ANA serogroup.

1. Anopheles A [Anopheles A, (CoAr 3624)*].

2. Lukuni [Lukuni].

3. (ColAn 57389)* [(ColAn 57389)*].

4. Tacaiuma [Tacaiuma, (CoAr 1071)*]. Varieties of (CoAr 1071)* virus: (CoAr 1071)*, (CoAr 3627)*.

The Anopheles B serogroup contains a single complex, Anopheles B, consisting of Anopheles B and Boraceia virus types.

The Bakau serogroup also contains a single complex, Bakau, consisting of Bakau and Ketapang virus types.

Viruses of the Mapputta group have been isolated only in Australia. SIRACA has designated three complexes for this serogroup, two containing a single virus type and the third containing two viruses. One of these two viruses is presently unregistered, but has been submitted for consideration.

1. Mapputta [Mapputta].

2. Maprik [Maprik, (Gan Gan)*].

3. Trubanaman [Trubanaman].

Two virus members of the Turlock group have been associated with birds.

SIRACA has assigned a single complex containing one virus type to this serogroup.

1. Turlock [Turlock]. Turlock virus subtypes: Turlock, Umbre, M'Poko.

Table 20. Minor antigenic groups of viruses: Members of these minor antigenic groups have been characterized and taxonomically classified as orbiviruses.

* ( ): Presently unregistered.

Several of the viruses in these minor antigenic groups are important in causing disease in large animals. Bluetongue virus causes disease in both wild and domestic ruminants; AHS in mules, donkeys, and horses; and EHD in deer. Recently, Bluetongue virus has been isolated from Culicoides in the Northern Territory, Australia, thus extending its geographic distribution to that region.

SEAS has upgraded the status of Corriparta virus from Possible Arbovirus to Probable Arbovirus.

D'Aguilar virus recently has been isolated repeatedly from a viremic cow.

The Wallal serogroup has been added to this list of minor antigenic groups. Australian workers have isolated an antigenic relative of Wallal virus from Culicoides marksi. Physicochemical and electron microscopic characterization of both viruses indicates that they are orbiviruses.

Table 21. Minor antigenic groups of viruses: Members of the serogroups listed in this table possess a "bullet-shaped" morphology and are classified as rhabdoviruses.

The Hart Park serogroup now consists of five virus members. Mosqueiro virus, from Culex mosquitoes collected in Brazil, recently was accepted for registration, and it was demonstrated that it is related by CF to both Hart Park virus and Kamese virus of the former Mossuril serogroup. Former members of the Mossuril group have been consolidated into the Hart Park antigenic group. All of the present members are associated with a mosquito vector and three of the viruses (Hart Park, Flanders, Mossuril) have been isolated from birds.

The Sawgrass group, consisting of the recently registered New Minto virus and Sawgrass virus, has been added to the list of minor antigenic groups listed in this table. Both viruses appear to be tick-associated and have been characterized by electron microscopy as being rhabdoviruses.

Timbo and Chaco viruses of the Timbo serogroup have not been associated with any vector thus far.

Thus far, only members of the VSV group have been implicated in causing human disease in nature.

Table 22. Minor antigenic groups of viruses: These antigenic groups consist of members which are taxonomically unclassified.

Both Marburg and Ebola viruses cause human disease in nature and have been associated with laboratory-acquired infections. Nyando virus has been isolated from a single case of febrile illness in man.

This table now includes the newly formed Tanjong Rabok serogroup consisting of Tanjong Rabok and the recently registered Telok Forest viruses.

Both viruses have been isolated in Malaysia and neither has been associated with a vector, although Telok Forest virus was isolated from a wild monkey and Tanjong Rabok virus from a sentinel monkey.

Table 23. Tacaribe group viruses: Tacaribe group viruses are serologically related to lymphocytic choriomeningitis virus, and they are classified taxonomically in the Arenavirus genus. They are primarily rodent viruses, and there is little or no evidence that they are associated with an arthropod vector in nature. SEAS has judged all members to be non-arthropod-borne.

Three members of this group have been implicated in severe, often fatal human disease. These include Junin (Argentine hemorrhagic fever), Machupo (Bolivian hemorrhagic fever), and Lassa (Lassa disease). In addition to causing overt laboratory-acquired infections, Junin virus also has been demonstrated to cause subclinical laboratory-acquired infections. A subclinical seroconversion to Tacaribe virus has been documented in a laboratory worker handling large quantities of Tacaribe virus.

Table 24. Ungrouped mosquito-associated viruses: The viruses in this table are serologically ungrouped, though they have been clustered together on the basis of their association with a mosquito vector and placed into subsets according to their taxonomic designation. Of those placed in the bunyavirus-like category, Rift Valley fever virus is best known. It causes serious and extensive disease in domestic animals such as sheep and cattle and may cause disease in veterinary personnel, field and laboratory workers, as well as herdsmen who handle infected animals.

Bocas virus was formerly included in the CAL group until it was demonstrated that it was identical to or closely related to mouse hepatitis virus.

The picornavirus, Nodamura, was isolated from wild-caught mosquitoes in Japan, and it has been demonstrated to produce disease in moths and honey bees. While it has been shown that it replicates in mosquitoes and is experimentally transmitted by mosquitoes, consideration should be given to the possibility that it represents a true or an evolved form of insect pathogen.

Three of the listed bunyavirus-like agents (RVF, TAT, ZGA), one orbivirus (ORU), and the poxvirus, Cotia, have caused human disease in nature. Lebombo virus, or a closely related virus, has been isolated from human plasma, although it has not been associated with the causation of disease in man thus far.

Table 25. Ungrouped mosquito-associated viruses: These serologically ungrouped viruses are associated with a mosquito vector but are taxonomically unclassified. The recently registered Rochambeau virus, isolated from mosquitoes collected in French Guiana, has been added to this list. Very little is known about this virus at the present.

Table 26. Ungrouped tick-, culicoides-, or phlebotomus-associated viruses: The serologically ungrouped viruses listed in Table 26 appear to be primarily associated with non-mosquito vectors. Approximately two-thirds of

the listed viruses are taxonomically unclassified. A laboratory-acquired infection with Bhanja virus has been documented. None of the other viruses have been implicated in causing human disease.

Three registered viruses (Khasan, Razdan, Tamdy), isolated in the USSR, have been added to the set of bunyavirus-like agents. The recently registered Chim virus, from ticks in the USSR; Inhangapa virus, from phlebotomine flies in Brazil; and Slovakia viruses, from ticks in Czechoslovakia, have been added to the list of taxonomically unclassified viruses shown in Table 26.

Issyk-Kul and Keterah viruses have been shown to be closely related or identical by CF. The decision to designate them as either the same virus or as antigenic relatives must await results of cross-neutralization testing. Pending that decision, these viruses are being listed in the ungrouped category.

Table 27. Ungrouped viruses, no arthropod vector known: None of the listed viruses have been isolated from an arthropod vector, and they are not rated higher than Possible Arbovirus. Several of the viruses are rated Probably not Arbovirus or Not Arbovirus.

Bangui and Le Dantec viruses have been isolated from man, and Bangui virus has been reported to cause a febrile illness with rash in man.

Approximately one-third of the viruses listed in Table 27 have been taxonomically classified.

Two recently registered viruses have been added to the list of taxonomically unclassified agents. Araguari virus was isolated from an opossum trapped in Brazil, while Sakpa virus was isolated from a rodent collected in the Central African Empire.

Table 28 gives continental distribution of viruses in different antigenic groups on the basis of virus isolation. Overall, the registered viruses are very limited in their distribution. Approximately 85% have been isolated on a single continent only, while 16 or 3.9% have been found on 3 or more continents. The largest number of viruses have been isolated in Africa.

Table 29 shows the number of viruses, according to antigenic group, which have been isolated from various classes of arthropods. Fifty percent have been recovered from mosquitoes, about 23% from ticks, and 14% from all other classes. Eighty-six (21%) registered viruses have never been recovered from any arthropod vector. The largest number of viruses which have been isolated from any arthropod have been recovered from a single class only (295 of 322, 92%).

Table 30 presents a similar type of analysis in terms of virus isolations from various classes of vertebrates. Man and rodents have provided the largest number of virus isolations. Most of the viruses isolated from vertebrates have been recovered from a single class only (173 of 246, 70%).

Table 31 lists the viruses in each antigenic group which cause disease in man. Approximately 25% of all registered viruses have been associated with human disease, either in nature, or by laboratory-acquired infections, or both. Members of group A, group B, and Bunyamwera Supergroup, which constitute 43% of all registered viruses, account for 68% of the instances of association of registered viruses with disease in man.

An analysis of the SEAS ratings for all registered viruses is presented in Table 32, and it shows that 216 (53%) registrations are rated as Possible Arboviruses. Clearly, additional data are required if we are to have a more precise rating of the arthropod-borne status of these viruses. Sufficient data are available for about 47% of all registered viruses so that 41% are rated Probable Arbovirus or Arbovirus, while 6% are rated Probably not Arbovirus or Not Arbovirus.

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Table 1

ALPHABETICAL LISTING OF 408 VIRUSES REGISTERED AS OF 31 DEC. 1978
WITH RECOMMENDED ABBREVIATIONS AND ANTIGENIC GROUPING

NAME	ABBR.	ANTI-GENIC GROUP
ABSETTAROV	ABS	B
ABU HAMMAD	AH	DGK
ACADO	ACD	COR
ACARA	ACA	CAP
AFRICAN HORSESICKNESS	AHS	AHS
AFRICAN SWINE FEVER	ASF	
AGUACATE	AGU	PHL
AINO	AINO	SIM
AKABANE	AKA	SIM
ALFUY	ALF	B
ALMPIWAR	ALM	
AMAPARI	AMA	TCR
ANHANGA	ANH	PHL
ANHEMBI	AMB	BUN
ANOPHELES A	ANA	ANA
ANOPHELES B	ANB	ANB
APEU	APEU	C
APOI	APOI	B
ARAGUARI	ARA	
ARIDE	ARI	
ARKONAM	ARK	
ARUAC	ARU	
ARUMOWOT	AMT	PHL
AURA	AURA	A
AVALON	AVA	SAK
BAGAZA	BAG	B
BAHIG	BAH	TETE
BAKAU	BAK	BAK
BAKU	BAKU	KEM
BANDIA	BDA	QYB
BANGORAN	BGN	
BANGUI	BGI	
BANZI	BAN	B
BARUR	BAR	
BATAI	BAT	BUN
BATAMA	BMA	TETE
BATKEN	BKN	
BATU CAVE	BC	B
BAULINE	BAU	KEM
BEBARU	BEB	A
BELMONT	BEL	
BERTIOGA	BER	GMA
BHANJA	BHA	
BIMBO	BBO	
BIMITI	BIM	GMA
BIRAO	BIR	BUN

NAME	ABBR.	ANTI-GENIC GROUP
BLUETONGUE	BLU	BLU
BOBAYA	BOB	
BOBIA	BIA	OLI
BOCAS	BOC	
BORACEIA	BOR	ANB
BOTAMBI	BOT	OLI
BOTEKE	BTK	BTK
BOUBOUI	BOU	B
BOVINE EPHEMERAL FEVER	BEF	
BUENAVENTURA	BUE	PHL
BUJARU	BUJ	PHL
BUNYAMWERA	BUN	BUN
BURG EL ARAB	BEA	MTY
BUSHBUSH	BSB	CAP
BUSSUQUARA	BSQ	B
BUTTONWILLOW	BUT	SIM
BWAMBA	BWA	BWA
CABASSOU	CAB	A
CACAO	CAC	PHL
CACHE VALLEY	CV	BUN
CAIMITO	CAI	PHL
CALIFORNIA ENC.	CE	CAL
CALOVO	CVO	BUN
CANDIRU	CDU	PHL
CAPE WRATH	CW	KEM
CAPIM	CAP	CAP
CARAPARU	CAR	C
CAREY ISLAND	CI	B
CATU	CATU	GMA
CHACO	CHO	TIM
CHAGRES	CHG	PHL
CHANDIPURA	CHP	VSV
CHANGUINOLA	CGL	CGL
CHARLEVILLE	CHV	
CHENUDA	CNU	KEM
CHIKUNGUNYA	CHIK	A
CHILIBRE	CHI	PHL
CHIM	CHIM	
CHOBAR GORGE	CG	
CLO MOR	CM	SAK
COCAL	COC	VSV
COLORADO TICK FEVER	CTF	CTF
CONGO	CON	CON
CORRIPARTA	COR	COR
COTIA	COT	
COWBONE RIDGE	CR	B
D'AGUILAR	DAG	PAL
DAKAR BAT	DB	B
DENGUE-1	DEN-1	B
DENGUE-2	DEN-2	B
DENGUE-3	DEN-3	B
DENGUE-4	DEN-4	B

NAME	ABBR.	ANTI-GENIC GROUP
DERA GHAZI KHAN	DGK	DGK
DHORI	DHO	
DUGBE	DUG	NSD
EAST. EQUINE ENC.	EEE	A
EBOLA	EBO	MBG
EDGE HILL	EH	B
ENTEBBE BAT	ENT	B
EP. HEM. DIS.	EHD	EHD
EUBENANGEE	EUB	EUB
EVERGLADES	EVE	A
EYACH	EYA	CTF
FLANDERS	FLA	HP
FORT MORGAN	FM	A
FRIJOLES	FRI	PHL
GAMBOA	GAM	SBU
GANJAM	GAN	NSD
GARBA	GAR	MTY
GERMISTON	GER	BUN
GETAH	GET	A
GOMOKA	GOM	
GORDIL	GOR	PHL
GOSSAS	GOS	
GRAND ARBAUD	GA	UUK
GREAT ISLAND	GI	KEM
GUAJARA	GJA	CAP
GUAMA	GMA	GMA
GUARATUBA	GTB	SBU
GUAROA	GRO	CAL
GUMBO LIMBO	GL	C
HANZALOVA	HAN	B
HART PARK	HP	HP
HAZARA	HAZ	CON
HUACHO	HUA	KEM
HUGHES	HUG	HUG
HYPR	HYPR	B
IBARAKI	IBA	
ICOARACI	ICO	PHL
IERI	IERI	
ILESHA	ILE	BUN
ILHEUS	ILH	B
INGWAVUMA	ING	SIM
INHANGAPI	INH	
ININI	INI	SIM
INKOO	INK	CAL
IPPY	IPPY	
IRITUIA	IRI	CGL
ISFAHAN	ISF	VSV
ISRAEL TURKEY MEN.	IT	B
ISSYK-KUL	IK	
ITAITUBA	ITA	PHL
ITAPORANGA	ITP	PHL
ITAQUI	ITQ	C

NAME	ABBR.	ANTI-GENIC GROUP
JAMESTOWN CANYON	JC	CAL
JAPANAUT	JAP	
JAPANESE ENC.	JE	B
JERRY SLOUGH	JS	CAL
JOHNSTON ATOLL	JA	QRF
JOINJAKAKA	JOI	
JUAN DIAZ	JD	CAP
JUGRA	JUG	B
JUNIN	JUN	TCR
JURONA	JUR	SBU
JUTIAPA	JUT	B
KADAM	KAD	B
KAENG KHOI	KK	SBU
KAIKALUR	KAI	SIM
KAIRI	KRI	BUN
KAISODI	KSO	KSO
KAMESE	KAM	HP
KAMMAVANPETTAI	KMP	
KANNAMANGALAM	KAN	
KAO SHUAN	KS	DGK
KARIMABAD	KAR	PHL
KARSHI	KSI	B
KASBA	KAS	PAL
KEMEROVO	KEM	KEM
KERN CANYON	KC	
KETAPANG	KET	BAK
KETERAH	KTR	
KEURALIBA	KEU	
KEYSTONE	KEY	CAL
KHASAN	KHA	
KLAMATH	KLA	
KOKOBERA	KOK	B
KOLONGO	KOL	
KOONGOL	KOO	KOO
KOUTANGO	KOU	B
KOWANYAMA	KOW	
KUMLINGE	KUM	B
KUNJIN	KUN	B
KUNUNURRA	KNA	
KWATTA	KWA	KWA
KYASANUR FOR. DIS.	KFD	B
KYZYLAGACH	KYZ	A
LA CROSSE	LAC	CAL
LAGOS BAT	LB	*
LA JOYA	LJ	
LANDJIA	LJA	
LANGAT	LGT	B
LANJAN	LJN	KSO
LASSA	LAS	TCR
LATINO	LAT	TCR
LEBOMBO	LEB	
LE DANTEC	LD	

*Rabies related

NAME	ABBR.	ANTI-GENIC GROUP
LIPOVNIK	LIP	KEM
LOKERN	LOK	BUN
LONE STAR	LS	
LOUPING ILL	LI	B
LUKUNI	LUK	ANA
MACHUPO	MAC	TCR
MADRID	MAD	C
MAGUARI	MAG	BUN
MAHOGANY HAMMOCK	MH	GMA
MAIN DRAIN	MD	BUN
MALAKAL	MAL	MAL
MANAWA	MWA	UUK
MANZANILLA	MAN	SIM
MAPPUTTA	MAP	MAP
MAPRIK	MPK	MAP
MARBURG	MBG	MBG
MARCO	MCO	
MARITUBA	MTB	C
MATARIYA	MTY	MTY
MATRUH	MTR	TETE
MATUCARE	MAT	
MAYARO	MAY	A
MELAO	MEL	CAL
MERMET	MER	SIM
MIDDELBURG	MID	A
MINATITLAN	MNT	MIR
MINNAL	MIN	
MIRIM	MIR	MIR
MITCHELL RIVER	MR	WAR
MODOC	MOD	B
MOJU	MOJU	GMA
MONO LAKE	ML	KEM
MONT. MYOTIS LEUK.	MML	B
MORICHE	MOR	CAP
MOSQUEIRO	MQO	HP
MOSSURIL	MOS	HP
MOUNT ELGON BAT	MEB	
M'POKO	MPO	TUR
MUCAMBO	MUC	A
MURRAY VALLEY ENC.	MVE	B
MURUTUCU	MUR	C
NAIROBI SHEEP DIS.	NSD	NSD
NARIVA	NAR	
NAVARRO	NAV	
NDUMU	NDU	A
NEGISHI	NEG	B
NEPUYO	NEP	C
NEW MINTO	NM	SAW
NGAINGAN	NGA	
NIQUE	NIQ	PHL
NKOLBISSON	NKO	
NODAMURA	NOD	

NAME	ABBR.	ANTI-GENIC GROUP
NOLA	NOLA	SIM
NORTHWAY	NOR	BUN
NTAYA	NTA	B
NUGGET	NUG	KEM
NYAMANINI	NYM	
NYANDO	NDO	NDO
OKHOTSKIY	OKH	KEM
OKOLA	OKO	
OLIFANTSVLEI	OLI	OLI
OMSK HEM. FEVER	OMSK	B
O'NYONG NYONG	ONN	A
ORIBOCA	ORI	C
OROPOUCHE	ORO	SIM
ORUNGO	ORU	
OSSA	OSSA	C
OUANGO	OUA	
OUBANGUI	OUB	
PACORA	PCA	
PACUI	PAC	PHL
PAHAYOKEE	PAH	PAT
PALYAM	PAL	PAL
PARAMUSHIR	PMR	
PARANA	PAR	TCR
PATA	PATA	EUB
PATHUM THANI	PTH	DGK
PATOIS	PAT	PAT
PHNOM-PENH BAT	PPB	B
PICHINDE	PIC	TCR
PIRY	PIRY	VSV
PIXUNA	PIX	A
PONGOLA	PGA	BWA
PONTEVES	PTV	UUK
POWASSAN	POW	B
PRETORIA	PRE	DGK
PUCHONG	PUC	MAL
PUNTA SALINAS	PS	HUG
PUNTA TORO	PT	PHL
QALYUB	QYB	QYB
QUARANFIL	QRF	QRF
RAZDAN	RAZ	
RESTAN	RES	C
RIFT VALLEY FEVER	RVF	
RIO BRAVO	RB	B
RIO GRANDE	RG	PHL
ROCHAMBEAU	RBU	
ROCIO	ROC	B
ROSS RIVER	RR	A
ROYAL FARM	RF	B
RUSS.SPR.SUM.ENC.	RSSE	B
SABO	SABO	SIM
SABOYA	SAB	B
SAGIYAMA	SAG	A

NAME	ABBR.	ANTI-GENIC GROUP
SAINT-FLORIS	SAF	
SAKHALIN	SAK	SAK
SAKPA	SPA	
SALANGA	SGA	
SALEHABAD	SAL	PHL
SAN ANGELO	SA	CAL
SANDFLY F. (NAPLES)	SFN	PHL
SANDFLY F. (SICILIAN)	SFS	PHL
SANDJIMBA	SJA	
SANGO	SAN	SIM
SANTA ROSA	SAR	BUN
SATHUPERI	SAT	SIM
SAUMAREZ REEF	SRE	B
SAWGRASS	SAW	SAW
SEBOKELE	SEB	
SELETAR	SEL	KEM
SEMBALAM	SEM	
SEMLIKI FOREST	SF	A
SEPIK	SEP	B
SERRA DO NAVIO	SDN	CAL
SHAMONDA	SHA	SIM
SHARK RIVER	SR	PAT
SHUNI	SHU	SIM
SILVERWATER	SIL	KSO
SIMBU	SIM	SIM
SIMIAN HEM. FEV.	SHF	
SINDBIS	SIN	A
SIXGUN CITY	SC	KEM
SLOVAKIA	SLO	
SNOWSHOE HARE	SSH	CAL
SOKOLUK	SOK	B
SOLDADO	SOL	HUG
SORONOCA	SOR	BUN
SPONDWENI	SPO	B
ST. LOUIS ENC.	SLE	B
STRATFORD	STR	B
SUNDAY CANYON	SCA	
TACAIUMA	TCM	ANA
TACARIBE	TCR	TCR
TAGGERT	TAG	SAK
TAHYNA	TAH	CAL
TAMDY	TDY	
TAMIAMI	TAM	TCR
TANGA	TAN	
TANJONG RABOK	TR	TR
TATAGUINE	TAT	
TELOK FOREST	TF	TR
TEMBE	TME	
TEMBUSU	TMU	B
TENSAW	TEN	BUN
TETE	TETE	TETE
TETTNANG	TET	

NAME	ABBR.	ANTI-GENIC GROUP
THIMIRI	THI	SIM
THOGOTO	THO	THO
THOTTAPALAYAM	TPM	
TIMBO	TIM	TIM
TLACOTALPAN	TLA	BUN
TONATE	TON	A
TOURE	TOU	
TRIBEC	TRB	KEM
TRINITI	TNT	
TRIVITTATUS	TVT	CAL
TRUBANAMAN	TRU	MAP
TSURUSE	TSU	TETE
TURLOCK	TUR	TUR
TYULENIY	TYU	B
UGANDA S	UGS	B
UMATILLA	UMA	
UMBRE	UMB	TUR
UNA	UNA	A
UPOLU	UPO	
URUCURI	URU	PHL
USUTU	USU	B
UUKUNIEMI	UUK	UUK
VELLORE	VEL	PAL
VEN. EQUINE ENC.	VEE	A
VENKATAPURAM	VKT	
VS-ALAGOAS	VSA	VSV
VS-INDIANA	VSI	VSV
VS-NEW JERSEY	VSNJ	VSV
WAD MEDANI	WM	KEM
WALLAL	WAL	WAL
WANOWRIE	WAN	
WARREGO	WAR	WAR
WESSELSBRON	WSL	B
WEST. EQUINE ENC.	WEE	A
WEST NILE	WN	B
WHATAROA	WHA	A
WITWATERSRAND	WIT	
WONGAL	WON	KOO
WONGORR	WGR	
WYEOMYIA	WYO	BUN
YAQUINA HEAD	YH	KEM
YATA	YATA	
YELLOW FEVER	YF	B
YOGUE	YOG	
ZALIV TERPENIYA	ZT	UUK
ZEGLA	ZEG	PAT
ZIKA	ZIKA	B
ZINGA	ZGA	
ZINGILAMO	ZGO	BTK
ZIRQA	ZIR	HUG

Table 2. Antigenic Groups of 408 Viruses Registered in Catalogue

Antigenic Group	Abbreviation	No. Registered Viruses in Group		%
A	A		24	5.9
African horsesickness	AHS		1	0.2
Anopheles A	ANA		3	0.7
Anopheles B	ANB		2	0.5
B	B		60	14.7
Bakau	BAK		2	0.5
Bluetongue	BLU		1	0.2
Boteke	BTK		2	0.5
Bunyamwera Supergroup			93	22.8
Bunyamwera Supergroup: Bunyamwera	BUN	18		
Bunyamwera Supergroup: Bwamba	BWA	2		
Bunyamwera Supergroup: C	C	11		
Bunyamwera Supergroup: California	CAL	13		
Bunyamwera Supergroup: Capim	CAP	6		
Bunyamwera Supergroup: Guama	GMA	6		
Bunyamwera Supergroup: Koongol	KOO	2		
Bunyamwera Supergroup: Mirim	MIR	2		
Bunyamwera Supergroup: Olifantsvlei	OLI	3		
Bunyamwera Supergroup: Patois	PAT	4		
Bunyamwera Supergroup: Simbu	SIM	17		
Bunyamwera Supergroup: Tete	TETE	5		
Bunyamwera Supergroup: Unassigned	SBU	4		
Changuinola	CGL		2	0.5
Colorado tick fever	CTF		2	0.5
Congo	CON		2	0.5
Corriparta	COR		2	0.5
Dera Ghazi Khan	DGK		5	1.2
Epizootic hemorrhagic disease	EHD		1	0.2
Eubenangee	EUB		2	0.5
Hart Park	HP		5	1.2
Hughes	HUG		4	1.0
Kaisodi	KSO		3	0.7
Kemerovo	KEM		16	3.9
Kwatta	KWA		1	0.2
Malakal	MAL		2	0.5
Mapputta	MAP		3	0.7
Marburg	MBG		2	0.5
Matariya	MTY		3	0.7
Nairobi sheep disease	NSD		3	0.7
Nyando	NDO		1	0.2
Palyam	PAL		4	1.0
Phlebotomus fever	PHL		24	5.9
Qalyub	QYB		2	0.5
Quaranfil	QRF		2	0.5
Sakhalin	SAK		4	1.0
Sawgrass	SAW		2	0.5
Tacaribe	TCR		9	2.2
Tanjong Rabok	TR		2	0.5
Thogoto	THO		1	0.2
Timbo	TIM		2	0.5
Turlock	TUR		3	0.7
Uukuniemi	UUK		5	1.2
Vesicular stomatitis	VSV		7	1.7
Wallal	WAL		1	0.2
Warrego	WAR		2	0.5
Ungrouped viruses			91	22.3
Total			408	

Table 3. Initial Isolations of Viruses by Decade and Country of Origin.

Decade	Continent	Country	Virus
1900-09	Africa	S. Africa	BLU
1910-19	Africa	Kenya	ASF,NSD
1920-29	Africa	Nigeria	YF
	Europe	Scotland	LI
	N. America	U.S.A.	VSI
1930-39	Africa	Kenya	RVF
		S. Africa	AHS
		Uganda	BWA,WN
	Asia	Japan	JE
		U.S.S.R.	RSSE
	N. America	U.S.A.	EEE,SLE,WEE
	S. America	Venezuela	VEE
1940-49	Africa	Uganda	BUN,NTA,SF,UGS,ZIKA
	Asia	Japan	NEG
		U.S.S.R.	OMSK
	Australasia	Hawaii	DEN-1*
		New Guinea	DEN-2*
	Europe	Czechoslovakia	HAN
		Italy	SFN*,SFS*
	N. America	U.S.A.	CE,CTF,TVT
	S. America	Brazil	ILH
		Colombia	ANA,ANB,WYO
1950-59	Africa	Egypt	CNU,QRF,QYB,SIN,WM
		Nigeria	ILE,LB
		S. Africa	BAN,GER,ING,LEB,MID,MOS,NDU,NYM, PGA,SIM,SPO,TETE,USU,WIT,WSL
		Uganda	CHIK,CON,ENT,NDO,ONN,ORU
	Asia	India	ARK,BHA,GAN,KAS,KSO,KFD,MIN,PAL, SAT,VKT,UMB,WAN
		Israel	IT
		Japan	AKA,APOI,IBA,NOD,SAG,TSU
		Malaya	BAK,BAT,BEB,GET,KET,LGT,TMU
	Australasia	Australia	MVE
		Philippines	DEN-3*,DEN-4*
	Europe	Czechoslovakia	HYPR,TAH
		Finland	KUM
		U.S.S.R	ABS
	N. America	Canada	POW
		Panama	BOC,LJ,PCA
		U.S.A.	CV,EHD,HP,MML,MOD,RB,SA,SSH,TUR,VSNJ
	S. America	Argentina	JUN
		Brazil	APEU,AURA,BSQ,CAP,CAR,CATU,GJA,GMA, ITQ,MAG,MIR,MOJU,MTB,MUC,MUR,ORI, TCM,UNA
		Colombia	GRO,NAV
		Trinidad	ARU,BIM,BSB,IERI,KRI,LUK,MAN,MAY, MEL,NEP,ORO,TCR,TNT

* Isolated in U.S.A. Laboratory

Table 3. (Continued)

Decade	Continent	Country	Virus
1960-69	Africa	Cameroon	NKO,OKO
		Cent.Afr.Emp.	BAG,BGN,BIA,BIR,BOT,BOU,BTK,MPO,PATA,YATA, ZGA
		Egypt	ACD,AMT,BAH*,BEA,MTR,MTY,RF
		Kenya	THO
		Nigeria	DUG,LAS*,SABO,SAN,SHA,SHU
		Senegal	BDA,DB,GOS,KEU,KOU,LD,SAB,TAT,TOU,YOG
		South Africa	OLI
		Sudan	MAL***
		Uganda	KAD,KAM,MEB,TAN
	Asia	Cambodia	PPB
		India	BAR,CHP,DHO,KAN,KMP,SEM,THI,TPM,VEL
		Iran	KAR*,SAL*
		Japan	AINO
		Malaysia	JUG,KTR,LJN,PUC,TR
		Pakistan (West)	DGK,HAZ,MWA
		Persian Gulf	ZIR
		Singapore	SEL
		Thailand	KK
		U.S.S.R.	KYZ,OKH,SAK,TYU,ZT
	Australasia	Australia	ALF,ALM,BEF,BEL,CHV,COR,DAG,EH,EUB,JAP, JOI,KOK,KOO,KOW,KUN,MAP,MPK,MR,RR,SEP, STR,TRU,UPO,WAR,WON
		New Zealand	WHA
		Pacific Island	JA*
	Europe	Czechoslovakia	CVO,KEM,LIP,TRB
		Finland	INK,UUK
		France	GA,PTV
		West Germany	MBG
	N. America	Canada	SIL
		Guatemala	JUT*
		Mexico	MNT,TLA*
		Panama	AGU,CHG,CHI,CGL,FRI,GAM,JD,LAT,MAD, MAT,OSSA,PAR,PAT,PT*,ZEG
		U.S.A.	BUT,CR,EVE,FLA,GL,HUG,JC,JS,KC,KEY,KLA, LAC,LOK,LS,MER,MD,MH,ML,PAH,SAW,SC,SHF, SR,TAM,TEN,UMA
	S. America	Bolivia	MAC**
		Brazil	ACA,AMA,AMB,ANH,ARA,BER,BOR,BUJ,CDU,CHO, COT,GTB,ICO,INH,IRI,ITP,JUR,MCO,PAC,PIRY, PIX,SDN,SOR,TIM,TME,URU,VSA
		Colombia	BUE,PIC
		French Guiana	CAB
		Peru	HUA*,PS*
		Surinam	KWA
		Trinidad	COC,MOR,NAR,RES,SOL

* Isolated in U.S.A. laboratory
** Isolated in Panama laboratory
*** Isolated in Egypt laboratory

Table 3. (Continued)

Decade	Continent	Country	Virus
1970-78	Africa	Cent.Afr.Emp.	BBO,BGI,BMA,BOB,GAR,GOM,GOR,IPPY,KOL,LJA, NOLA,OUA,OUB,SAF,SEB,SGA,SJA,SPA,ZGO,
		Egypt	AH,KS,PTH
		Seychelles	ARI***
		S. Africa	PRE***
		Zaire	EBO
	Asia	India	CG,KAI
		Iran	ISF*
		Malaysia	BC,CI,TF
		U.S.S.R.	BKN,CHIM,IK,KHA,KSI,PMR,RAZ,SOK,TDY
	Australasia	Australia	KNA,NGA,NUG,SRE,TAG,WAL,WGR
	Europe	Czechoslovakia	SLO
		Germany	EYA,TET
		Scotland	CM,CW
		U.S.S.R.	BAKU
	N. America	Canada	AVA,BAU*,GI*
		Mexico	SAR*
		Panama	CAC,CAI,NIQ
		U.S.A.	FM,NM,NOR,RG,SCA,YH
	S. America	Brazil	ITA,MQO,ROC
		French Guiana	INI,RBU,TON

* Isolated in U.S.A. laboratory
** Isolated in Panama laboratory
*** Isolated in Egypt laboratory

Table 4. Initial Isolation of 408 Registered Viruses by Continent, Country, and Chronological Period.

Continent	Country or Area	Before 1930	1930 -39	1940 -49	1950 -59	1960 -69	1970 -78	Totals
AFRICA	Cameroon					2		2
	Cent.Afr.Emp.					11	19	30
	Egypt				5	7	3	15
	Kenya	2	1			1		4
	Nigeria	1			2	6		9
	Senegal					10		10
	Seychelles						1	1
	S. Africa	1	1		15	1	1	19
	Sudan					1		1
	Uganda		2	5	6	4		17
	Zaire						1	1
	Totals	4	4	5	28	43	25	109
ASIA	Cambodia					1		1
	India				12	9	2	23
	Iran					2	1	3
	Israel				1			1
	Japan		1	1	6	1		9
	Malaysia				7	5	3	15
	W. Pakistan					3		3
	Persian Gulf					1		1
	Singapore					1		1
	Thailand					1		1
	U.S.S.R.(East)		1	1		5	9	16
	Totals	0	2	2	26	29	15	74
AUSTRAL-	Australia				1	25	7	33
ASIA	Hawaii			1				1
and	Johnston Island					1		1
PACIFIC	New Guinea			1				1
ISLANDS	New Zealand					1		1
	Philippines				2			2
	Totals	0	0	2	3	27	7	39
EUROPE	Czechoslovakia			1	2	4	1	8
	Finland				1	2		3
	France					2		2
	West Germany					1	2	3
	Italy			2				2
	Scotland	1					2	3
	U.S.S.R.(West)				1		1	2
	Totals	1	0	3	4	9	6	23
NORTH	Canada				1	1	3	5
AMERICA	Guatemala					1		1
	Mexico					2	1	3
	Panama				3	15	3	21
	U.S.A.	1	3	3	10	26	6	49
	Totals	1	3	3	14	45	13	79
SOUTH	Argentina				1			1
AMERICA	Bolivia					1		1
	Brazil			1	18	27	3	49
	Colombia			3	2	2		7
	French Guiana					1	3	4
	Peru					2		2
	Surinam					1		1
	Trinidad				13	5		18
	Venezuela		1					1
	Totals	0	1	4	34	39	6	84
	Grand Totals	6	10	19	109	192	72	408

Table 5. Group A Arboviruses

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Aura	+														+					+			22	Alphavirus
Bebaru	+																+						22	"
Cabassou	+										+	+	+		+		+				+		21	"
Chikungunya	+							+	+		+	+			+	+	+				+	+	20	"
Eastern equine enc.	+	+				+	+	+	+	+	+		+	+			+	+	+	+	+	+	20	"
Everglades	+	+								+			+							+			20	"
Fort Morgan							+				+									+			20	"
Getah	+	+												+			+	+					20	"
Kyzylagach	+																+						22	"
Mayaro	+						+	+						+							+	+	20	"
Middelburg	+															+							20	"
Mucambo	+							+		+	+		+		+						+	+	20	"
Ndumu	+													+		+							21	"
O'nyong-nyong		+						+		+					+	+						+	20	"
Pixuna	+	+								+											+		22	"
Ross River	+							+			+		+					+				+	20	"
Sagiyama	+																+						21	"
Semliki Forest	+	+						+			+					+	+					+	20	"
Sindbis	+	+					+	+			+					+	+	+			+		20	"
Tonate	+	+						+			+										+		21	"
Una	+	+												+						+	+		21	"
Venezuelan equine enc.	+	+						+		+		+	+	+						+	+	+	20	"
Western equine enc.	+	+					+	+		+	+			+					?	+	+	+	20	"
Whataroa	+																	+					20	"

* 20 = Arbovirus
21 = Probable Arbovirus
22 = Possible Arbovirus
23 = Probably not Arbovirus
24 = Not Arbovirus

Table 6. Group B Arboviruses, Mosquito-Borne

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Alfuy	+										+							+						20	Flavivirus
Bagaza	+															+								22	*
Banzi	+							+							+	+						+		20	*
Bouboui	+	+							+							+								22	*
Bussuquara	+							+		+					+					+	+	+		20	*
Dengue-1	+							+								+	+	+				+	+	20	*
Dengue-2	+							+	+							+	+	+			+	+	+	20	*
Dengue-3	+							+									+	+			+	+	+	20	*
Dengue-4	+							+									+	+				+		20	*
Edge Hill	+	+																+						20	*
Ilheus	+							+			+				+					+	+	+	+	20	*
Japanese encephalitis	+	+						+			+	+		+			+					+	+	20	*
Jugra	+											+					+							22	*
Kokobera	+																	+						21	*
Kunjin	+							+			+							+					+	20	*
Murray Valley enc.	+							+										+				+		20	*
Ntaya	+															+								21	*
Sepik	+																	+				+		21	*
St. Louis encephalitis	+	+					+	+			+	+		+						+	+	+	+	20	*
Spondweni	+							+								+						+	+	20	*
Stratford	+																	+						22	*
Tembusu	+	+													+		+	+						21	*
Uganda S	+										+				+	+								20	*
Usutu	+									+	+					+								22	*
Wesselsbron	+	+						+		+				+		+	+					+	+	20	*
West Nile	+	+	+	+				+		+	+	+		+	+	+	+		+			+	+	20	*
Yellow fever	+							+	+				+			+					+	+	+	20	*
Zika	+							+							+	+	+					+	+	20	*

* See footnote Table 5

Table 7. Group B Arboviruses, Tick-Borne

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Absettarov			+					+						+					+			+	+	20	Flavivirus
Hanzalova			+					+						+					+			+		20	"
Hypr			+					+		+	+	+		+					+			+	+	20	"
Kadam			+					+								+								21	"
Karshi				+						+	+						+							22	"
Kumlinge			+					+		+	+			+					+			+	+	20	"
Kyasanur Forest disease			+	+			+	+	+	+	+	+		+			+					+	+	20	"
Langat			+														+							20	"
Louping ill			+					+		+	+			+					+			+	+	20	"
Omsk hem. fev.			+					+		+				+			+		?			+	+	20	"
Powassan			+					+		+				+						+		+		20	"
Royal Farm				+													+							22	"
RSSE			+					+		+	+						+		+			+	+	20	"
Saumarez Reef			+	+														+						22	"
Tyuleniy			+														+			+				21	"

* See footnote Table 5

Table 8. Group B Viruses, No Arthropod Vector Demonstrated

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Apoi										+							+						+	22	Flavivirus
Batu Cave												+					+							22	"
Carey Island												+					+							22	"
Cowbone Ridge										+										+				23	"
Dakar bat								+				+				+								24	"
Entebbe bat												+				+								24	"
Israel turkey meningo.											+						+							21	"
Jutiapa										+										+				22	"
Koutango										+						+							+	21	"
Modoc										+										+				24	"
Montana myotis leuko.												+								+				24	"
Negishi								+									+					+	+	22	"
Phnom-Penh bat												+					+							23	"
Rio Bravo												+								+		+	+	24	"
Rocio								+			+										+	+	+	22	"
Saboya										+						+								22	"
Sokuluk												+					+							22	"

* See footnote Table 5

Table 9. Bunyamwera Supergroup: Bunyamwera Group Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Anhembi	+									+										+				22	Bunyavirus
Batai	+	+															+		+					21	"
Birao		+														+								22	"
Bunyamwera	+							+								+						+	+	20	"
Cache Valley	+	+																		+				20	"
Calovo**		+																	+			+	+	21	"
Germiston	+							+		+					+	+							+	20	"
Ilesha		+						+								+						+		21	"
Kairi	+								+	+					+						+			20	"
Lokern	+					+								+	+					+	+			20	"
Maguari	+	+				+								+	+					+	+			20	"
Main Drain						+								+	+					+				20	"
Northway	+														+					+				21	"
Santa Rosa	+																			+				22	"
Sororoca	+																				+			22	"
Tensaw	+	+												+	+					+		+		20	"
Tlacotalpan	+	+																		+				22	"
Wyeomyia	+	+						+												+	+	+		21	"

* See footnote Table 5
** May be strain of Batai

Table 10. Bunyamwera Supergroup: Bwamba Group and Group C Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
BWAMBA GROUP																									
Bwamba		+						+								+						+		21	Bunyavirus
Pongola	+	+														+								20	"
GROUP C																									
Apeu	+							+					+		+						+	+	+	20	Bunyavirus
Caraparu	+							+		+					+					+	+	+		20	"
Gumbo Limbo	+							+		+					+					+				21	"
Itaqui	+							+		+			+		+						+	+		20	"
Madrid	+							+		+					+					+		+		20	"
Marituba	+							+					+		+						+	+		20	"
Murutucu	+							+		+			+	+	+						+	+		20	"
Nepuyo	+							+		+		+			+					+				20	"
Oriboca	+							+		+			+		+						+	+	+	20	"
Ossa	+							+		+					+					+		+		20	"
Restan	+							+													+	+		20	"

* See footnote Table 5

Table 11. Bunyamwera Supergroup: California and Capim Group Viruses

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks																					
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
CALIFORNIA GROUP																									
California enc.	+																			+		+		20	Bunyavirus
Guaroa		+						+												+	+	+ +		20	"
Inkoo	+																		+			+ +		22	"
Jamestown Canyon	+						+								+					+				20	"
Jerry Slough	+																			+				20	"
Keystone	+	+								+					+					+				20	"
La Crosse	+							+		+					+					+		+		20	"
Melao	+																				+			21	"
San Angelo		+																		+				22	"
Serra do Navio	+																				+			22	"
Snowshoe Hare	+									+				+	+					+				20	"
Tahyna	+	+						+							+	+	+		+			+		20	"
Trivittatus	+									+					+					+				20	"
CAPIM GROUP																									
Acara	+									+					+					+	+			21	Bunyavirus
Bushbush	+														+						+			20	"
Capim	+									+			+		+					+	+			20	"
Guajara	+									+					+					+	+			20	"
Juan Diaz															+					+				22	"
Moriche	+																				+			22	"

* See footnote Table 5

Table 12. Bunyamwera Supergroup: Guama, Koongol, Mirim, Olifantsvlei, and Patois Group Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks																						
	Culicine	Anopheline	Ixodid	Argasid	Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
GUAMA GROUP																									
Bertioga															+						+			22	Bunyavirus
Bimiti	+									+					+						+			20	"
Catu	+	+						+		+		+	+		+						+	+		20	"
Guama	+				+			+		+		+	+		+					+	+	+		20	"
Mahogany Hammock	+									+										+				22	"
Moju	+									+			+		+						+			20	"
KOONGOL GROUP																									
Koongol	+	?																+						21	Bunyavirus
Wongal	+																	+						21	"
MIRIM GROUP																									
Minatitlan															+					+				22	Bunyavirus
Mirim	+														+					·	+			20	"
OLIFANTSVLEI GROUP																									
Botambi	+															+								22	Bunyavirus
Bobia	+															+								22	"
Olifantsvlei	+															+								22	"
PATOIS GROUP																									
Pahayokee	+																			+				22	Bunyavirus
Patois	+									+					+					+				20	"
Shark River	+	+								+					+					+				21	"
Zegla										+					+					+				22	"

* See footnote Table 5

Table 13. Bunyamwera Supergroup: Simbu Group Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Aino	+	+				+								+			+	+						22	Bunyavirus
Akabane	+					+								+			+	+						21	"
Buttonwillow						+														+				20	"
Ingwavuma	+										+			+		+	+				+			20	"
Inini											+										+			22	"
Kaikalur	+																+				+			22	"
Manzanilla									+												+			22	"
Mermet											+									+				22	"
Nola	+																							20	"
Oropouche	+							+						+		+					+	+		21	"
Sabo						+								+		+								22	"
Sango	+					+								+		+								22	"
Sathuperi	+					+								+		+	+							22	"
Shamonda																+								22	"
Shuni	+					+		+						+		+						+		22	"
Simbu	+															+								21	"
Thimiri											+					+	+							22	"

* See footnote Table 5

Table 14. Bunyamwera Supergroup: Tete Group and Unassigned Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
TETE GROUP																									
Bahig			+								+					+			+					21	Bunyavirus
Batama											+					+								22	"
Matruh			+								+					+			+					22	"
Tete											+					+								22	"
Tsuruse											+						+							22	"
UNASSIGNED - "SBU"																									
Gamboa	+																			+				22	Bunyavirus
Guaratuba	+										+				+						+			21	"
Jurona	+																				+			22	"
Kaeng Khoi							+					+			+		+							22	"

* See footnote Table 5

Table 15. Phlebotomus Fever Group Viruses

VIRUS	ISOLATED FROM																ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																		
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																						
Aguacate					+															+				21	Bunyavirus-like
Anhanga														+							+			22	"
Arumowot	+									+				+		+								22	"
Buenaventura					+															+	+			22	"
Bujaru										+											+			22	"
Cacao					+															+				21	"
Caimito					+															+				22	"
Candiru								+													+	+		22	"
Chagres	+				+			+												+		+		21	"
Chilibre					+															+				21	"
Frijoles					+															+				22	"
Gordil										+						+								22	"
Icoaraci	+	+			+					+	+				+						+			21	"
Itaituba													+								+			22	"
Itaporanga	+										+		+		+						+			20	"
Karimabad					+												+							22	"
Nique					+															+				22	"
Pacui					+					+											+			21	"
Punta Toro					+			+												+	+	+		21	"
Rio Grande										+										+				22	"
Saint-Floris										+						+								22	"
Salehabad					+												+							22	"
SF-Naples					+			+								+	+		+			+		20	"
SF-Sicilian					+			+								+	+		+			+		20	"
Urucuri										+											+			22	"

* See footnote Table 5

Table 16. Tick-Borne Groups Other Than Group B Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
CHF-CONGO GROUP																									
Congo			+			+		+						+		+	+		+			+	+	20	Bunyavirus-like
Hazara			+														+							22	"
KAISODI GROUP																									
Kaisodi			+								+						+							22	Bunyavirus-like
Lanjan			+						+								+							22	"
Silverwater			+											+						+				21	"
NAIROBI SHEEP DISEASE																									
Dugbe	+		+			+		+		+				+		+						+		22	Bunyavirus-like
Ganjam	+		+					+									+					+	+	22	"
Nairobi sheep disease			+					+						+		+						+	+	20	"
SAKHALIN GROUP																									
Avalon			+								+									+				21	Bunyavirus-like
Clo Mor			+																+					22	"
Sakhalin			+														+			+				22	"
Taggert			+															+						22	"
THOGOTO GROUP																									
Thogoto			+					+						+		+			+			+		22	Bunyavirus-like
UUKUNIEMI GROUP																									
Grand Arbaud				+															+					20	Bunyavirus-like
Manawa			+	+													+							22	"
Ponteves				+															+					22	"
Uukuniemi			+							+	+								+					21	"
Zaliv Terpeniya			+														+							22	"

* See footnote Table 5

Table 17. Tick-Borne Groups Other Than Group B Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
COLORADO TICK FEVER																									
Colorado tick fever			+	+				+		+				+						+		+	+	20	Orbivirus
Eyach			+																+					22	"
KEMEROVO																									
Baku				+															+					22	Orbivirus
Bauline			+																	+				22	"
Cape Wrath			+																+					22	"
Chenuda				+												+								22	"
Great Island			+																	+				22	"
Huacho				+																	+			22	"
Kemerovo			+					+			+					+	+		+			+	+	21	"
Lipovnik			+																+					22	"
Mono Lake				+																+				22	"
Nugget			+															+						22	"
Okhotskiy			+														+							22	"
Seletar			+														+							22	"
Sixgun City				+																+				22	"
Tribec			+							+					+				+					21	"
Wad Medani			+													+	+			+				22	"
Yaquina Head			+																	+				22	"

* See footnote Table 5

Table 18. Tick-Borne Groups Other Than Group B Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
DERA GHAZI KHAN GROUP																									
Abu Hammad				+												+	+							22	Unclassified
Dera Ghazi Khan			+	+													+ +							22	"
Kao Shuan				+													+ +	+						22	"
Pathum Thani				+ +													+ +							22	"
Pretoria				+												+								22	"
HUGHES GROUP																									
Hughes				+							+									+	+			21	Unclassified
Punta Salinas				+																	+			22	"
Soldado				+												+			+		+			20	"
Zirqa				+													+							22	"
QALYUB GROUP																									
Bandia				+						+						+								22	Unclassified
Qalyub				+												+								22	"
QUARANFIL GROUP																									
Johnston Atoll				+							+							+						20	Unclassified
Quaranfil				+				+			+					+						+		20	"

* See footnote Table 5

Table 19. Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
ANOPHELES A GROUP																									
Anopheles A		+																		+			21	Bunyavirus-like
Lukuni	+	+																		+			22	"
Tacaiuma	+	+						+							+					+			21	"
ANOPHELES B GROUP																									
Anopheles B		+																		+			22	Bunyavirus-like
Boraceia	+	+																		+			22	"
BAKAU GROUP																									
Bakau	+			+					+								+						22	Bunyavirus-like
Ketapang	+																+						21	"
MAPPUTTA GROUP																									
Mapputta		+																+					22	Bunyavirus-like
Maprik	+	+																+					21	"
Trubanaman		+																+					22	"
TURLOCK GROUP																									
M'Poko (=Yaba-1)	+										+			+	+	+					+		22	Bunyavirus-like
Turlock	+										+			+	+					+	+		20	"
Umbre	+										+						+						21	"

* See footnote Table 5

Table 20. Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
AFRICAN HORSESICKNESS																								
African horsesickness						+								+		+	+		+					20	Orbivirus
BLUETONGUE GROUP																								
Bluetongue						+								+		+	+	+	+	+				20	Orbivirus
CHANGUINOLA GROUP																								
Changuinola					+			+												+		+		21	Orbivirus
Irituia										+											+			22	"
CORRIPARTA GROUP																								
Acado	+															+								22	Orbivirus
Corriparta	+										+							+						21	"
EHD GROUP																								
Epizootic hem. dis.														+						+				21	Orbivirus
EUBENANGEE GROUP																								
Eubenangee	+?	+?																+						22	Orbivirus
Pata	+															+								22	"
PALYAM GROUP																								
D'Aguilar						+								+				+						22	Orbivirus
Kasba	+																+							22	"
Palyam	+																+							22	"
Vellore	+																+							22	"
WALLAL GROUP																								
Wallal						+												+						22	Orbivirus
WARREGO GROUP																								
Mitchell River						+												+						22	Orbivirus
Warrego						+												+						22	"

* See footnote Table 5

Table 21. Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
HART PARK GROUP																									
Flanders	+										+									+				22	Rhabdovirus
Hart Park	+										+									+				21	"
Kamese	+															+								22	"
Mosqueiro	+																				+			22	"
Mossuril	+										+					+								22	"
KWATTA GROUP																									
Kwatta	+																				+			22	Rhabdovirus
SAWGRASS GROUP																									
New Minto			+																	+				22	Rhabdovirus
Sawgrass			+																	+				22	"
TIMBO GROUP																									
Chaco														+							+			22	Rhabdovirus
Timbo														+							+			22	"
VESICULAR STOMATITIS GR.																									
Chandipura					+			+						+	+	+	+					+		20	Rhabdovirus
Cocal	+						+			+				+	+						+			20	"
Isfahan					+												+							22	"
Piry								+					+								+		+	22	"
VS-Alagoas														+							+	+		22	"
VS-Indiana	+				+			+						+						+	+	+	+	20	"
VS-New Jersey							+	+						+						+	+	+	+	22	"

* See footnote Table 5

Table 22. Minor Antigenic Groups of Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
BOTEKE GROUP																									
Boteke	+															+								22	Unclassified
Zingilamo											+					+								22	"
MALAKAL GROUP																									
Malakal	+															+								22	Unclassified
Puchong	+																+							22	"
MARBURG GROUP																									
Ebola								+								+						+	+	23	Unclassified
Marburg								+								+			+			+	+	23	"
MATARIYA GROUP																									
Burg el Arab											+					+								22	Unclassified
Garba											+					+								22	"
Matariya											+					+								22	"
NYANDO GROUP																									
Nyando		+						+								+						+		21	Unclassified
TANJONG RABOK GROUP																									
Tanjong Rabok															+		+							22	Unclassified
Telok Forest									+								+							22	"

* See footnote Table 5

Table 23. Tacaribe (LCM) Group Viruses

VIRUS	ISOLATED FROM														ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentine:s	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Amapari							+	+		+											+			24	Arenavirus
Junin							+	+		+				+		+					+	+	+	24	"
Lassa								+		+											+	+	+	24	"
Latino										+											+	+	+	24	"
Machupo										+											+	+	+	24	"
Parana			+				+			+											+		+	24	"
Pichinde	?	?								+		+								+	+		+	24	"
Tacaribe																									
Tamiami																									

* See footnote Table 5

Table 24. Ungrouped Mosquito-Associated Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Belmont	+							+								+		+		·				22	Bunyavirus-like
Kowanyama		+																+						22	"
Rift Valley fever	+							+						+		+						+	+	20	"
Tataguine	+	+						+								+						+		21	"
Triniti	+																				+			21	"
Witwatersrand	+									+					+	+								20	"
Zinga	+							+								+						+		22	"
Bocas	+											+								+	+			22	Coronavirus
Japanaut	+											+						+						21	Orbivirus
Lebombo	+						+	+		+						+								21	"
Orungo	+	+														+						+		21	"
Umatilla	+										+									+				20	"
Nodamura	+																+							23	Picornavirus
Cotia	+				+			+							+						+	+		24	Poxvirus
Joinjakaka	+																	+						22	Rhabdovirus
Kununurra	+																	+						22	"
Yata	+															+								22	"

* See footnote Table 5

Table 25. Ungrouped Mosquito-Associated Viruses

VIRUS	ISOLATED FROM																ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																		
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																						
Arkonam	+	+															+				+			22	Unclassified
Aruac	+																				+			21	*
Bangoran	+										+					+								22	*
Gomoka	+	+									+					+								22	*
Ieri	+																				+			22	*
La Joya	+																			+				22	*
Minnal	+																+							22	*
Nkolbisson	+															+								22	*
Okola	+															+								22	*
Oubangui	+															+								22	*
Pacora	+																			+				22	*
Rochambeau	+																				+			22	*
Tanga		+														+					+			22	*
Tembe		+																			+			22	*
Venkatapuram	+																+							22	*
Wongorr	+																	+						22	*

* See footnote Table 5

Table 26. Ungrouped Tick-, Culicoides-, or Phlebotomus-Associated Viruses

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Bhanja			+					+		+				+		+	+		+				+	22	Bunyavirus-like
Khasan			+														+							22	"
Lone Star			+																	+				22	"
Razdan			+														+							22	"
Sunday Canyon				+																+				22	"
Tamdy				+													+							22	"
African swine fever				+										+		+			+		+**			20	Iridovirus
Barur			+							+							+							22	Rhabdovirus
Bovine ephemeral fever						+								+		+	+	+						22	"
Aride			+													+								22	Unclassified
Batken	+		+														+							22	"
Charleville					+									+				+						22	"
Chim			+														+							22	"
Chobar Gorge				+													+							22	"
Dhori			+													+	+							22	"
Inhangapi					+																+			22	"
Issyk-Kul				+								+					+							22	"
Keterah				+								+					+							21	"
Matucare				+																	+			22	"
Ngaingan						+												+						22	"
Nyamanini				+							+					+								21	"
Paramushir			+														+							22	"
Slovakia				+															+					22	"
Tettnang			+																+					22	"
Upolu				+														+						22	"
Wanowrie	+		+					+								+	+							22	"

* See footnote Table 5
** Cuba

Table 27. Ungrouped Viruses: No Arthropod Vector Known

VIRUS	ISOLATED FROM															ISOLATED IN						HUMAN DISEASE		SEAS RATING*	TAXONOMIC STATUS
	ARTHROPODS							VERTEBRATES																	
	Mosq.		Ticks		Phlebotomine	Culicoides	Other	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Other	Sentinels	Africa	Asia	Australasia	Europe	North America	South America	Natural Infection	Lab Infection		
	Culicine	Anopheline	Ixodid	Argasid																					
Bobaya											+					+								22	Bunyavirus-like
Ibaraki														+			+							22	Orbivirus
Nariva										+											+			23	Paramyxovirus
Kern Canyon												+								+				23	Rhabdovirus
Keuraliba										+						+								22	"
Klamath										+										+				22	"
Lagos bat												+				+								24	"
Marco														+							+			22	"
Mount Elgon bat												+				+								23	"
Navarro											+										+			22	"
Almpiwar														+				+						21	Unclassified
Araguari													+								+			22	"
Bangui								+								+						+		22	"
Bimbo											+					+								22	"
Gossas												+				+								23	"
Ippy										+						+								22	"
Kammavanpettai											+						+							22	"
Kannamangalm											+						+							22	"
Kolongo											+					+								22	"
Landjia											+					+								22	"
Le Dantec								+								+								22	"
Ouango											+					+								22	"
Sakpa										+						+								22	"
Salanga										+						+								22	"
Sandjimba											+					+								22	"
Sebokele										+						+								22	"
Sembalam											+						+							22	"
Simian hem. fever									+								?			+				24	"
Thottapalayam														+			+							22	"
Toure										+						+								22	"
Yogue												+				+								22	"

* See footnote Table 5

Table 28. Continental Distribution of Grouped and Ungrouped Viruses.

Antigenic Group	Total in Group	Africa	Asia	Australasia	Europe	North America	South America	No. of Continents involved 1	2	3	4	5	6
A	24	6	8	5	1	6	10	16	6	1	0	1	0
AHS	1	1	1	0	1	0	0	0	0	1	0	0	0
ANA	3	0	0	0	0	0	3	3	0	0	0	0	0
ANB	2	0	0	0	0	0	2	2	0	0	0	0	0
B	60	18	24	13	7	10	7	46	10	3	1	0	0
BAK	2	0	2	0	0	0	0	2	0	0	0	0	0
BLU	1	1	1	1	1	1	0	0	0	0	0	0	1
BTK	2	2	0	0	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup BUN	18	4	1	0	2	8	5	16	2	0	0	0	0
Bunyamwera Supergroup BWA	2	2	0	0	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup C	11	0	0	0	0	5	8	9	2	0	0	0	0
Bunyamwera Supergroup CAL	13	1	1	0	2	9	3	11	1	1	0	0	0
Bunyamwera Supergroup CAP	6	0	0	0	0	3	5	4	2	0	0	0	0
Bunyamwera Supergroup GMA	6	0	0	0	0	2	5	5	1	0	0	0	0
Bunyamwera Supergroup KOO	2	0	0	2	0	0	0	2	0	0	0	0	0
Bunyamwera Supergroup MIR	2	0	0	0	0	1	1	2	0	0	0	0	0
Bunyamwera Supergroup OLI	3	3	0	0	0	0	0	3	0	0	0	0	0
Bunyamwera Supergroup PAT	4	0	0	0	0	4	0	4	0	0	0	0	0
Bunyamwera Supergroup SIM	17	9	6	2	0	2	3	12	5	0	0	0	0
Bunyamwera Supergroup TETE	5	4	1	0	2	0	0	3	2	0	0	0	0
Bunyamwera Supergroup SBU	4	0	1	0	0	1	2	4	0	0	0	0	0
CGL	2	0	0	0	0	1	1	2	0	0	0	0	0
CTF	2	0	0	0	1	1	0	2	0	0	0	0	0
CHF-CON	2	1	2	0	1	0	0	1	0	1	0	0	0
COR	2	1	0	1	0	0	0	2	0	0	0	0	0
DGK	5	2	4	1	0	0	0	3	2	0	0	0	0
EHD	1	0	0	0	0	1	0	1	0	0	0	0	0
EUB	2	1	0	1	0	0	0	2	0	0	0	0	0
HP	5	2	0	0	0	2	1	5	0	0	0	0	0
HUG	4	1	1	0	1	1	3	2	1	1	0	0	0
KSO	3	0	2	0	0	1	0	3	0	0	0	0	0
KEM	16	3	4	1	4	6	1	14	1	1	0	0	0
KWA	1	0	0	0	0	0	1	1	0	0	0	0	0
MAL	2	1	1	0	0	0	0	2	0	0	0	0	0
MAP	3	0	0	3	0	0	0	3	0	0	0	0	0
MBG	2	2	0	0	0	0	0	2	0	0	0	0	0
MTY	3	3	0	0	0	0	0	3	0	0	0	0	0
NSD	3	2	1	0	0	0	0	3	0	0	0	0	0
NDO	1	1	0	0	0	0	0	1	0	0	0	0	0
PAL	4	0	3	1	0	0	0	4	0	0	0	0	0
PHL	24	4	4	0	2	10	10	20	2	2	0	0	0
QYB	2	2	0	0	0	0	0	2	0	0	0	0	0
QRF	2	1	0	1	0	0	0	2	0	0	0	0	0
SAK	4	0	1	1	1	2	0	3	1	0	0	0	0
SAW	2	0	0	0	0	2	0	2	0	0	0	0	0
TCR	9	1	0	0	0	1	7	9	0	0	0	0	0
THO	1	1	0	0	1	0	0	0	1	0	0	0	0
TIM	2	0	0	0	0	0	2	2	0	0	0	0	0
TR	2	0	2	0	0	0	0	2	0	0	0	0	0
TUR	3	1	1	0	0	1	1	2	1	0	0	0	0
UUK	5	0	2	0	3	0	0	5	0	0	0	0	0
VSV	7	1	2	0	0	2	5	4	3	0	0	0	0
WAL	1	0	0	1	0	0	0	1	0	0	0	0	0
WAR	2	0	0	2	0	0	0	2	0	0	0	0	0
Ungrouped	91	39	24	11	4	9	14	85	3	3	0	0	0
Totals	408	121	100	47	34	92	100	345	46	14	1	1	1

Table 29. Number of Viruses Isolated From Wild Caught Arthropods

Antigenic Group	Total in Group	Isolated From: Mosq.	Ticks	Phlebotomine Flies	Culicoides	Other	No. of Classes Involved: 1	2	3
A	24	23	0	0	1	5	20	3	1
AHS	1	0	0	0	1	0	1	0	0
ANA	3	3	0	0	0	0	3	0	0
ANB	2	2	0	0	0	0	2	0	0
B	60	28	16	0	0	2	40	3	0
BAK	2	2	1	0	0	0	1	1	0
BLU	1	0	0	0	1	0	1	0	0
BTK	2	1	0	0	0	0	1	0	0
Bunyamwera Supergroup: BUN	18	17	0	0	2	0	17	1	0
Bunyamwera Supergroup: BWA	2	2	0	0	0	0	2	0	0
Bunyamwera Supergroup: C	11	11	0	0	0	0	11	0	0
Bunyamwera Supergroup: CAL	13	13	0	0	0	1	12	1	0
Bunyamwera Supergroup: CAP	6	5	0	0	0	0	5	0	0
Bunyamwera Supergroup: GMA	6	5	0	1	0	0	4	1	0
Bunyamwera Supergroup: KOO	2	2	0	0	0	0	2	0	0
Bunyamwera Supergroup: MIR	2	1	0	0	0	0	1	0	0
Bunyamwera Supergroup: OLI	3	3	0	0	0	0	3	0	0
Bunyamwera Supergroup: PAT	4	3	0	0	0	0	3	0	0
Bunyamwera Supergroup: SIM	17	10	0	0	8	0	8	5	0
Bunyamwera Supergroup: TETE	5	0	2	0	0	0	2	0	0
Bunyamwera Supergroup: SBU	4	3	0	0	0	1	4	0	0
CGL	2	0	0	1	0	0	1	0	0
CTF	2	0	2	0	0	0	2	0	0
CHF-CON	2	0	2	0	1	0	1	1	0
COR	2	2	0	0	0	0	2	0	0
DGK	5	0	5	0	0	0	5	0	0
EHD	1	0	0	0	0	0	0	0	0
EUB	2	2	0	0	0	0	2	0	0
HP	5	5	0	0	0	0	5	0	0
HUG	4	0	4	0	0	0	4	0	0
KSO	3	0	3	0	0	0	3	0	0
KEM	16	0	16	0	0	0	16	0	0
KWA	1	1	0	0	0	0	1	0	0
MAL	2	2	0	0	0	0	2	0	0
MAP	3	3	0	0	0	0	3	0	0
MBG	2	0	0	0	0	0	0	0	0
MTY	3	0	0	0	0	0	0	0	0
NSD	3	2	3	0	1	0	1	1	1
NDO	1	1	0	0	0	0	1	0	0
PAL	4	3	0	0	1	0	4	0	0
PHL	24	4	0	15	0	0	15	2	0
QYB	2	0	2	0	0	0	2	0	0
QRF	2	0	2	0	0	0	2	0	0
SAK	4	0	4	0	0	0	4	0	0
SAW	2	0	2	0	0	0	2	0	0
TCR	9	1	1	0	0	3	3	1	0
THO	1	0	1	0	0	0	1	0	0
TIM	2	0	0	0	0	0	0	0	0
TR	2	0	0	0	0	0	0	0	0
TUR	3	3	0	0	0	0	3	0	0
UUK	5	0	5	0	0	0	5	0	0
VSV	7	2	0	3	0	2	3	2	0
WAL	1	0	0	0	1	0	1	0	0
WAR	2	0	0	0	2	0	2	0	0
Ungrouped	91	35	23	3	2	1	56	4	0
Totals	408	200	94	23	21	15	295	26	2

Table 30. Number of Viruses Isolated From Naturally Infected Vertebrates

Antigenic Group	Total in Group	Man	Other Primates	Rodents	Birds	Bats	Marsupials	Livestock	All Others	Number of Classes Involved					
										1	2	3	4	5	6
A	24	10	2	6	10	3	6	6	3	7	4	2	3	1	1
AHS	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
ANA	3	1	0	0	0	0	0	0	0	1	0	0	0	0	0
ANB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B	60	28	4	17	15	14	1	5	6	27	7	5	4	2	1
BAK	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
BLU	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
BTK	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
Bunyamwera Supergroup: BUN	18	4	1	3	0	0	0	1	3	9	2	0	0	0	0
Bunyamwera Supergroup: BWA	2	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Bunyamwera Supergroup: C	11	10	0	8	0	1	5	0	1	2	5	3	1	0	0
Bunyamwera Supergroup: CAL	13	3	0	4	0	0	0	0	1	4	2	0	0	0	0
Bunyamwera Supergroup: CAP	6	0	0	3	0	0	1	0	0	2	1	0	0	0	0
Bunyamwera Supergroup: GMA	6	2	0	5	0	2	3	0	0	2	1	0	2	0	0
Bunyamwera Supergroup: KOO	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup: MIR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup: OLI	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bunyamwera Supergroup: PAT	4	0	0	3	0	0	0	0	0	3	0	0	0	0	0
Bunyamwera Supergroup: SIM	17	2	1	0	4	0	0	6	2	10	3	0	0	0	0
Bunyamwera Supergroup: TETE	5	0	0	0	5	0	0	0	0	5	0	0	0	0	0
Bunyamwera Supergroup: SBU	4	0	0	0	1	1	0	0	0	2	0	0	0	0	0
CGL	2	1	0	1	0	0	0	0	0	2	0	0	0	0	0
CTF	2	1	0	1	0	0	0	0	0	0	0	1	0	0	0
CHF-CON	2	1	0	0	0	0	0	1	1	0	1	0	0	0	0
COR	2	0	0	0	1	0	0	0	0	1	0	0	0	0	0
DGK	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EHD	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
EUB	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HP	5	0	0	0	3	0	0	0	0	3	0	0	0	0	0
HUG	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
KSO	3	0	1	0	1	0	0	0	1	3	0	0	0	0	0
KEM	16	1	0	1	1	0	0	0	0	1	1	0	0	0	0
KWA	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAL	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MAP	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
MBG	2	2	0	0	0	0	0	0	0	2	0	0	0	0	0
MTY	3	0	0	0	3	0	0	0	0	3	0	0	0	0	0
NSD	3	3	0	1	0	0	0	1	1	1	1	1	0	0	0
NDO	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0
PAL	4	0	0	0	0	0	0	1	0	1	0	0	0	0	0
PHL	24	5	0	7	2	0	2	0	2	12	3	0	0	0	0
QYB	2	0	0	1	0	0	0	0	0	1	0	0	0	0	0
QRF	2	1	0	0	1	0	0	0	0	0	1	0	0	0	0
SAK	4	0	0	0	1	0	0	0	0	1	0	0	0	0	0
SAW	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TCR	9	3	0	8	0	1	0	0	1	6	2	1	0	0	0
THO	1	1	0	0	0	0	0	1	0	0	1	0	0	0	0
TIM	2	0	0	0	0	0	0	0	2	2	0	0	0	0	0
TR	2	0	1	0	0	0	0	0	0	1	0	0	0	0	0
TUR	3	0	0	0	2	0	0	0	1	1	1	0	0	0	0
UUK	5	0	0	1	1	0	0	0	0	0	1	0	0	0	0
VSV	7	4	0	1	0	0	1	4	1	1	5	0	0	0	0
WAL	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
WAR	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ungrouped	91	9	1	13	14	9	1	6	3	49	2	1	0	0	0
Totals	408	94	12	84	67	31	20	34	30	173	44	14	10	3	2

Table 31. Number of Viruses Associated with Naturally or Laboratory Acquired Disease in Man.

Antigenic Group		Total in Group	In Nature	Lab. Infection	Either or Both	
					Number	Percent
Group A		24	10	8	12	50.0
Afr. horsesickness		1	0	0	0	
Anopheles A		3	0	0	0	
Anopheles B		2	0	0	0	
Group B		60	28	24	31	52.0
Bakau		2	0	0	0	
Bluetongue		1	0	0	0	
Boteke		2	0	0	0	
Bunyamwera Supergroup	Bunyamwera	18	5	2	6	33.3
	Bwamba	2	1	0	1	50.0
	C	11	9	2	9	81.8
	California	13	5	0	5	38.5
	Capim	6	0	0	0	
	Guama	6	2	0	2	33.3
	Koongol	2	0	0	0	
	Mirim	2	0	0	0	
	Olifantsvlei	3	0	0	0	
	Patois	4	0	0	0	
	Simbu	17	2	1	2	11.8
	Tete	5	0	0	0	
	SBU	4	0	0	0	
Changuinola		2	1	0	1	50.0
Colorado tick fever		2	1	1	1	50.0
CHF-Congo		2	1	1	1	50.0
Corriparta		2	0	0	0	
Dera Ghazi Khan		5	0	0	0	
Epizoot. hem. dis.		1	0	0	0	
Eubenangee		2	0	0	0	
Hart Park		5	0	0	0	
Hughes		4	0	0	0	
Kaisodi		3	0	0	0	
Kemerovo		16	1	1	1	6.3
Kwatta		1	0	0	0	
Malakal		2	0	0	0	
Mapputta		3	0	0	0	
Marburg		2	2	2	2	100.0
Matariya		3	0	0	0	
Nairobi sheep dis.		3	3	2	3	100.0
Nyando		1	1	0	1	100.0
Palyam		4	0	0	0	
Phlebotomus fever		24	5	0	5	20.8
Qalyub		2	0	0	0	
Quaranfil		2	1	0	1	50.0
Sakhalin		4	0	0	0	
Sawgrass		2	0	0	0	
Tacaribe		9	3	5	5	55.6
Tanjong Rabok		2	0	0	0	
Thogoto		1	1	0	1	100.0
Timbo		2	0	0	0	
Turlock		3	0	0	0	
Uukuniemi		5	0	0	0	
Vesicular stom.		7	4	3	5	71.4
Wallal		1	0	0	0	
Warrego		2	0	0	0	
Ungrouped		91	6	2	7	7.7
Totals		408	92	54	102	25.0

Table 32. Evaluation of Arthropod-Borne Status of 408 Registered Viruses (SEAS)

Antigenic Group	Total in Group	Arbovirus	Probably Arbovirus	Possible Arbovirus	Probably not Arbovirus	Not Arbovirus	Arbo or Probably Arbo		Not or Probably not Arbo	
							No.	%	No.	%
A	24	15	5	4	0	0	20	83.0	0	
AHS	1	1	0	0	0	0	1	100.0	0	
ANA	3	0	2	1	0	0	2	66.7	0	
ANB	2	0	0	2	0	0	0		0	
B	60	29	8	16	2	5	37	61.7	7	11.7
BAK	2	0	1	1	0	0	1	50.0	0	
BLU	1	1	0	0	0	0	1	100.0	0	
BTK	2	0	0	2	0	0	0		0	
Bunyamwera Supergroup: BUN	18	8	5	5	0	0	13	72.2	0	
Bunyamwera Supergroup: BWA	2	1	1	0	0	0	2	100.0	0	
Bunyamwera Supergroup: C	11	10	1	0	0	0	11	100.0	0	
Bunyamwera Supergroup: CAL	13	9	1	3	0	0	10	76.9	0	
Bunyamwera Supergroup: CAP	6	3	1	2	0	0	4	66.7	0	
Bunyamwera Supergroup: GMA	6	4	0	2	0	0	4	66.7	0	
Bunyamwera Supergroup: KOO	2	0	2	0	0	0	2	100.0	0	
Bunyamwera Supergroup: MIR	2	1	0	1	0	0	1	50.0	0	
Bunyamwera Supergroup: OLI	3	0	0	3	0	0	0		0	
Bunyamwera Supergroup: PAT	4	1	1	2	0	0	2	50.0	0	
Bunyamwera Supergroup: SIM	17	3	3	11	0	0	6	35.3	0	
Bunyamwera Supergroup: TETE	5	0	1	4	0	0	1	20.0	0	
Bunyamwera Supergroup: SBU	4	0	1	3	0	0	1	25.0	0	
CGL	2	0	1	1	0	0	1	50.0	0	
CTF	2	1	0	1	0	0	1	50.0	0	
CHF-CON	2	1	0	1	0	0	1	50.0	0	
COR	2	0	1	1	0	0	1	50.0	0	
DGK	5	0	0	5	0	0	0		0	
EHD	1	0	1	0	0	0	1	100.0	0	
EUB	2	0	0	2	0	0	0		0	
HP	5	0	1	4	0	0	1	50.0	0	
HUG	4	1	1	2	0	0	2	50.0	0	
KSO	3	0	1	2	0	0	1	33.3	0	
KEM	16	0	2	14	0	0	2	12.5	0	
KWA	1	0	0	1	0	0	0		0	
MAL	2	0	0	2	0	0	0		0	
MAP	3	0	1	2	0	0	1	33.3	0	
MBG	2	0	0	0	2	0	0		2	100.0
MTY	3	0	0	3	0	0	0		0	
NSD	3	1	0	2	0	0	1	33.3	0	
NDO	1	0	1	0	0	0	1	100.0	0	
PAL	4	0	0	4	0	0	0		0	
PHL	24	3	7	14	0	0	10	41.7	0	
QYB	2	0	0	2	0	0	0		0	
QRF	2	2	0	0	0	0	2	100.0	0	
SAK	4	0	1	3	0	0	1	25.0	0	
SAW	2	0	0	2	0	0	0		0	
TCR	9	0	0	0	0	9	0		9	100.0
THO	1	0	0	1	0	0	0		0	
TIM	2	0	0	2	0	0	0		0	
TR	2	0	0	2	0	0	0		0	
TUR	3	1	1	1	0	0	2	66.7	0	
UUK	5	1	1	3	0	0	2	40.0	0	
VSV	7	3	0	4	0	0	3	42.9	0	
WAL	1	0	0	1	0	0	0		0	
WAR	2	0	0	2	0	0	0		0	
Ungrouped	91	4	9	70	5	3	13	14.3	8	8.8
Totals	408	104	62	216	9	17	166	40.7	26	6.4

REPORT FROM THE ARBOVIRUS RESEARCH UNIT

EGYPTIAN ORGANIZATION FOR BIOLOGICAL AND VACCINE PRODUCTION

AGOUZA, CAIRO, EGYPT

I. Serological Survey for Certain Tickborne Viruses of Potential Public Health Importance.

A. Bhanja Virus :

Bhanja (BHA) virus was originally isolated from Haemaphysalis intermedia (= H. parva) ticks taken from a paralysed Beetal goat in 1954 at Bhanjanagar, Orissa, India (Shah and Work, 1969). Subsequent studies resulted in isolating BHA virus from 8 other tick species in Asia, Europe, and Africa: Haemaphysalis punctata (Italy, Yugoslavia, Bulgaria), H. sulcata (Bulgaria), Boophilus decoloratus (Nigeria, Cameroun), Amblyomma variegatum (Senegal, Nigeria, Central African Empire), Hyalomma truncatum (Nigeria, Senegal), H. marginatum turanicum (Kirgiz SSR), H. detritum (Kazakh SSR), Dermacentor marginatus (Armenian SSR), and Rhipicephalus bursa (Azerbaijan SSR). BHA virus was also isolated from blood of cattle, sheep, ground squirrels, and hedgehogs in Nigeria. Serological studies have showed the presence of antibodies to BHA virus in goats (India, Italy, Czechoslovakia), sheep (Nigeria, Italy, Yugoslavia, Bulgaria, Czechoslovakia), horses (India), cattle (Nigeria, Italy), humans (India, Nigeria, Italy, Yugoslavia, Armenian SSR), rodents and the gundi

(Mus, Gerbillus, Apodemus, Ctenodactylus in Tunisia), and migratory birds (brambling finch, hawfinch, and blackbird in Italy). Three accidental, laboratory-acquired infections in humans were reported by Calisher and Goodpasture (1975) and Punda et al. (1978). A BHA virus infection in a seriously ill human in Yugoslavia was reported by Vesenjak-Hirjan et al. (1978).

In the first survey for the presence of Bhanja (BHA) virus in Egypt, 2,593 sera (433 from humans, 2,160 from domestic animals and rats) were investigated by the hemagglutination-inhibition (HI) and complement-fixation (CF) tests. HI antibodies to BHA virus were not detected in human and horse sera but were detected in sera from the donkey, sheep, goat, buffalo, cow, camel, pig, and Rattus rattus (Table 1). The antibody prevalence rates were between ca. 3 and 18 percent of the sera investigated. CF antibody prevalence was lower than that of HI antibodies. HI titers were 1:10 to 1:80; those of CF tests were 1:4 to 1:32.

The presence of antibodies to BHA virus in Egyptian sera extends the known distribution of this virus from western and central Africa to Egypt in northeastern Africa. Egypt is geographically situated between numerous BHA foci in Eurasia (India, USSR, Iran, Bulgaria, Yugoslavia, Czechoslovakia, Italy), and tropical Africa (Senegal, Nigeria, Cameroun, Central African Empire).

Rattus rattus and the domestic buffalo, camel, and donkey have not previously been implicated in BHA virus circulation.

Nine tick species from which BHA virus has been isolated elsewhere are listed above. Certain of these species are common in Egypt. Hyalomma marginatum is common on domestic animals and the African and European subspecies of H. marginatum are carried to Egypt by birds migrating northward from Africa in the spring and southward from Eurasia in the fall. Hyalomma truncatum occurs in the southeastern coastal area of Egypt. Amblyomma variegatum is carried to Egypt on imported cattle and camels and by northward migrating birds. Boophilus annulatus is a potential BHA virus vector in Egypt. The potential for infected ticks to be introduced to Egypt by migrating birds and on imported sheep, camels, and cattle should be investigated, as well as the nature of BHA virus epidemiology and the risk to human health in Egypt, where numerous cases of "fever of unknown origin" remain undiagnosed.

The finding that the HI test gave higher prevalence rates than the CF test may be owing to the transient nature of CF antibodies, a phenomenon shown in experimentally infected mice (Klisenko and Shanoyan, 1975), which is presently being investigated.

Table 1. Antibodies to Bhanja Virus in Sera from Egypt

Sera	No. tested	HI antibodies No.	HI antibodies %	CF antibodies No.	CF antibodies %
Human	433	0	0	0	0
Horse	16	0	0	0	0
Donkey	61	2	3.28	1	1.64
Sheep	531	22	4.14	5	0.94
Goat	646	21	3.25	8	1.24
Buffalo	124	9	7.25	6	4.84
Cow	421	12	2.85	3	0.71
Calf	18	3	16.66	0	0
Camel	78	14	17.94	3	3.85
Pig	120	10	8.33	0	0
Rattus	145	16	11.03	ND*	-
TOTAL	2,593	109	4.2	26	1.00

*ND = not done.

B. Prevalence of Complement-fixing (CF) Antibodies to Four Tickborne Viruses :

Four tickborne viruses which are known to cause human disease, or are prime candidates as disease-causing, were compared for prevalence of their CF antibodies in animals in Egypt. These viruses, which are antigenically unrelated, are Dugbe (DUG), Crimean-Congo hemorrhagic fever (CCHF), Bhanja (BHA), and Tettnang (TET); the first three have never been reported from Egypt and the fourth was isolated only once from Hyalomma dromedarii ticks taken from camels at Baragil (near Cairo).

A total of 2,079 animal sera were examined by CF test for antibodies against the four viruses (Table 2). The domestic animal sera, were from abattoirs at cities representing Upper and Lower Egypt Governorates; horses were from special farm near Cairo and donkeys from markets in different Governorates.

Antibodies to each of the four viruses were detected in sera from sheep, buffalo, cattle, goat, and camel. Antibodies to DUG and TET viruses were also detected in sera from calf; two donkeys exhibited antibodies to TET and BHA viruses, respectively. Horses and pigs were free from CF antibodies to any of the four viruses. CF titers ranged from 1:4 to 1:32, the highest (avg) were those of BHA and CCHF viruses (Table 3). The percentage of overall positive reactions were 3.56% for DUG, 1.87% for TET, 1.78% for CCHF, and 1.25% for BHA.

Antibodies to CCHF virus were detected in sera from camel, goat, cow, buffalo and sheep. Egypt is geographically situated between many CCHF foci in Eurasia and Africa, where 27 species and subspecies of ticks have been incriminated as CCHF virus vectors. Six of these vector species are common members of the Egyptian tick fauna (Hyalomma anatolicum anatolicum, H. marginatum rufipes, H. impeltatum, Rhipicephalus sanguineus, R. turanicus and Boophilus annulatus). During the spring and fall passages, northward and southward migrating birds transport ticks through Egypt from within the African and Eurasian ranges of CCHF virus.

The presence of antibodies to DUG virus in sera from goat, buffalo, camel, cow, and sheep in Egypt also extends its known distribution from western and eastern Africa (Ethiopian Faunal Region) to Egypt in northeastern Africa (Palearctic Faunal Region). The buffalo and camel have not previously been implicated in DUG virus circulation. DUG virus has been isolated from several tick species in Senegal, Nigeria, Central African Empire, Cameroun, Uganda, and Ethiopia. Of the 10 tick species associated with DUG virus in Africa, Hyalomma marginatum rufipes, H. impeltatum, and Boophilus annulatus are common in Egypt, H. truncatum occurs in the southeastern coastal area, and Amblyomma variegatum and A. lepidum are introduced on camels and cattle and by migrating birds. DUG virus has also been isolated from febrile humans in Nigeria and Central African Empire and from cattle, sheep and goats.

Antibodies to the virus were found in human sera (3.5%) in Ibadan, Nigeria.

The detection of antibodies to BHA virus in sera from sheep, buffalo, cow, goat, and camel provide the first evidence of circulation of this virus in Egypt and elsewhere in northern Africa. Egypt is geographically situated between numerous Eurasian and African foci. The buffalo, camel, and donkey have not previously been implicated in circulation of BHA virus. Two tick species from which BHA virus had been isolated exist in Egypt (Hyalomma marginatum, H. truncatum) and Amblyomma variegatum and Boophilus annulatus may be vectors in Egypt.

TET virus was first isolated in 1970 from Ixodes ricinus in West Germany and from a pool (ArT 1147) of Hyalomma dromedarii from Baragil, Egypt. The possible relationship of this virus with tickborne meningopolyneuritis, erythema chronicum migrans, and Lyme arthritis has been suggested. The detection of antibodies against TET virus in sera from donkey, cow, camel, goat, buffalo, and sheep should stimulate us to broaden the scope of the study to determine whether this virus presents a risk to human health in Egypt.

These four tickborne viruses, may present a threat to human health in Egypt. Antibodies against them were detected in sera of the common domestic animals in Egypt. TET virus was isolated from a tick in

Egypt, but there had been no previous evidence of CCHF, DUG, or BHA virus circulation in Egypt or elsewhere in North Africa. Several of the tick vectors of these 3 viruses (DUG, CCHF, and BHA) are common in the Egyptian fauna. These results provide impetus for more intensive epidemiological investigations of these viruses in Egypt and also for developing and/or applying more sensitive serological tests.

Table 2

Complement-fixing antibodies against certain tickborne viruses in animal sera from Egypt

Sera	No. tested	DUG		TET		CCHF		BHA	
		No.	%	No.	%	No.	%	No.	%
Sheep	594	25	4.21	12	2.02	7	1.18	5	0.84
Buffalo	124	9	7.25	6	4.84	11	8.87	6	4.84
Cow	421	14	3.33	13	3.09	4	0.95	3	0.71
Goat	646	22	3.41	7	1.08	14	2.17	8	1.24
Camel	78	3	3.85	3	3.8	1	1.28	3	3.85
Calf	19	1	5.25	1	5.25	-	-	-	-
Horse	16	-	-	-	-	-	-	-	-
Donkey	77	-	-	1	1.30	-	-	1	1.30
Pig	120	-	-	-	-	-	-	-	-
Total	2,079	74	3.56	39	1.87	37	1.78	26	1.25

DUG = Dugbe

TET = Tettnang

CCHF = Crimean-Congo hemorrhagic fever

BHA = Bhanja

CF titers of positive sera for certain tickborne viruses in Egypt

Table 3

CF titer	DUG	TET	CCHF	BHA
1:4	40	24	22	13
1:8	25	12	14	8
1:16	9	3	-	3
1:32	-	-	1	2
1:64	-	-	-	-
Total	74	39	37	26

DUG = Dugbe CCHF = Crimean-Congo hemorrhagic fever

TET = Tettnang BHA = Bhanja

II. Epidemic of Rift Valley fever in Egypt*

In October 1977, a Rift Valley Fever (RVF) outbreak occurred in Sharqiya Governorate, northeast of Cairo. The disease was explosive and presented mainly as an acute febrile, dengue-like illness of man; however, severe cases complicated by hemorrhage, jaundice, meningo-encephalitis and retinitis were also encountered. This epidemic represents the first incidence in this country, and probably anywhere in North Africa, of RVF virus. RVF as reported from Kenya, Uganda and South Africa is considered to be an enzootic disease with occasional epidemic extension into man. The outbreak of Egypt on the contrary appeared first in human

and in an explosive manner. Another peculiarity for that epidemic was the relatively high number of severe complicated cases and in fact the first reports from Sharqiya Governorate described fatal cases with diffuse hemorrhage and liver failure.

The first human cases in the outbreak were observed on 28 September 1977 at Inshas, Sharqiya (Fig. 1). The disease spread rapidly, to more than 21 villages in Sharqiya Governorate, extending few weeks later to Qalyubiya and Giza Governorates of the Nile Delta. Later (about the 11 December 1977) cases appeared in Upper Egypt (Minya and Asyut Governorates). By the end of December 1977, the human epidemic was over. Official data showed about 18,000 cases, with 598 deaths, a C.F.R. of 3.3% (Table 4). In 80% of deaths with hemorrhagic manifestations, a pre-existing advanced bilharzial hepatosplenomegaly was noted. The C.F.R. of the disease among military personnel was only 0.2% which is possibly a reflection of the younger age, better health, accurate reporting and early medical care for that group. The disease incidence in humans had been explosive and in any one community reached a peak within 2-3 weeks. The longest period of an outbreak within a community was 6 weeks and the highest incidence rate was 21.5%. The disease attacked all ages and both sexes, with a higher incidence in adult males.

At the beginning of the outbreak, the diagnosis of RVF was carried out by virus isolation and/or serologically. RVF virus was isolated from

the serum of 53 of 56 clinically suspected cases and also from 4 blood-containing stool specimens and two throat washings (Table 5). Table 6 shows the seroconversion exhibited by a number of hospitalized patients.

Since it is very unusual for RVF outbreaks to appear first in humans, the situation in the animals was explored. Search proved that death and abortions had occurred weeks or months before the human epidemic, but passed unnoticed or were misdiagnosed. Teams were later sent to farms in different governorates upon notification of suspected cases and specimens were brought for virological studies. RVF virus was isolated during the epidemic from 27 sheep, and from a cow, a camel, a goat, a horse and a rat (Rattus rattus) (Table 7). The isolation of virus from camel and horse represent the first records from these animals.

A retrospective serologic study was carried out on sera stored in our freezers from previous years and collected for different purposes. The survey included sera from man and from different animal species distributed over many governorates representing geographically most of the country. The HI test was used to detect RVF antibodies. The results showed absence of RVF antibodies in about 6,000 serum specimens representing years 1972 to 1976 (Table 8). The earliest indication of animal infection was in April 1977 (6 months before the human epidemic) for sheep, buffalo, cow, and goat sera collected from the coastal Matruh, Alexandria and Port Said Governorates. Sera collected in late September

and early October 1977 (just before the Sharqiya human epidemic exploded) from domestic animals in Qena and Aswan Governorates in Upper Egypt also showed RVF antibodies.

The overall positive rates for 4,304 domestic animal sera collected in 1977 and to October 1978 was 16.4% ranging from 33.6% in sheep to 1.1% for donkey with other animals in between (Table 9). RVF antibodies in animal sera were detected in at least 15 Governorates representing both Upper and Lower Egypt ranging from about 5-70%. For the human serologic study, no sera were available from 1977 prior to the outbreak. The average positive reaction for 2,529 human sera collected from October 1977 - October 1978 was 19.5%. The lowest incidence of positives was noticed in the coastal Governorates (Matruh, Alexandria and Suez) with a north-to-south increase, a situation similar to what was previously reported for WN virus and attributed to variation in mosquito density (Tables 10 & 11).

The low incidence at the coastal governorates, the north-to-south increase in positive reactions, together with the termination of the epidemic after insecticidal spray can be circumstantial evidence for RVF being mosquito or vector-borne. In this respect the 4 isolates which we obtained from partially engorged Culex pipiens are being very cautiously interpreted in respect to incriminating a specific vector.

The 1977 outbreak in Egypt represents the first record of RVF in a North African country. A retrospective serologic study on about

6,000 human and animal sera showed that the virus was not present in Egypt before 1977. The fact that RVF epizootics, together with human cases, had occurred in Sudan, South to Egypt in 1973 and 1976 may suggest that the virus was introduced through Sudan. Egypt has not imported sheep or cattle from Sudan for quite a number of years. Some speculate that the virus virus was introduced to Egypt from Sudan through camels, which avoid the Aswan quarantine, along the Eastern desert and thus explain the outbreak in Sharqiya (an Eastern Governorate) which is famous for active trade in camels and for consumption of camel meat (Fig. 1). If one has to be confined to the idea of Sudan and camels, the situation in Libya should be thoroughly explored and investigated since we know of camels crossing the desert between Sudan and the bordering Sebha Governorate of Libya. This in turn might explain RVF antibodies detected for the first time in animal sera collected in April 1977 at Matruh and Alexandria Governorates of Egypt. Anyhow there are more suggestions to the question of how the virus was introduced to Egypt and why Sharqiya Governorate in particular?

Concerning the transmission of RVF virus, it is considered on epidemiological grounds that Culex pipiens plays an important role. The isolation of the virus from engorged mosquitoes suggests its role whether mechanically and/or biologically. The high-titered viremia in human patients can make one think of other vectors (as fleas, bedbugs) being involved in mechanical transmission. The isolation of the virus from

throat washings and feces of patients may also indicate another possible mode of man-to-man transmission. Infection in humans also resulted from contact with infected blood and meat. These combined different modes of transmission may be responsible for the explosive nature of the Sharqiya human outbreak. On the basis of epidemiological and experimental evidence it is considered that direct transmission between animals can play an important role.

In late June 1978, human cases of RVF reappeared in Sharqiya Governorate, but at different localities (Fig. 2). The virus was isolated and identified as RVF from 28 patients, 1 sheep, and 2 engorged Culex pipiens mosquitoes. The number of human cases of RVF, officially reported from late June 1978 till the 10th of October 1978 was 114 cases with 12 deaths, all from Sharqiya Governorate (C.F.R. of 10.5%). The predominating clinical picture was the febrile syndrome, with few fatal cases exhibiting hemorrhages and liver failure. Reports indicate that clinical RVF in animals appeared in 3 new Governorates which were supposed to be free (Damietta, Kafr El Sheikh and Gharbiya).

Such recurrence might be related to overwintering of the virus. In this case, the reservoir for RVF virus can be domestic animals as sheep and camel, or rodents, or vectors. A proven efficient vector may act as a reservoir just by overwintering or by transovarial transmission.

Among the much needed aspects for investigation is a country-wide survey for the prevalence of antibodies among man and animals, to

determine the still available virgin areas. Mild and/or inapparent cases together with under-reporting should not be belittled. In fact the recurrence of human cases of RVF were at Sharqiya Governorate, but in new localities with low herd immunity. The immunity resulting from clinical and subclinical infections should be studied. Another important aspect for study is the nature of the Egyptian virus isolate as compared to the prototype and to the Sudanese strain. This might give a clue to the way the virus was introduced to Egypt and it may also help to answer the question of the different clinical picture encountered in Egypt.

The immediate challenge is to prevent, if possible, RVF from being endemic in the country. Vaccination of livestock should be an immediate measure in that direction.

(Medhat A. Darwish and Imam Z. Imam)

*Presented at the 27th Annual Meeting of the American Society of Tropical Medicine & Hygiene, held in Chicago, Illinois, November 5-10, 1978.

Table 4

Clinical syndromes of RVF in man in Egypt (1977 epidemic)

	Clinical syndrome	Main clinical features
1)	Acute febrile dengue-like illness	Sudden onset of high fever, headache, prostration, pain in the back, extremities and joints. Duration 3 days followed by recovery or complications.
2)	Hemorrhagic complications	Diffuse hemorrhage, liver failure, ending usually into coma and death.
3)	Ocular complications	Visual disturbance in one or both eyes, macular exudate, prognosis variable.
4)	Meningoencephalitic complications	Meningismus, hallucination generally a slow recovery.

Table 5

Isolation of RVF virus from specimens from patients clinically diagnosed as RVF at "fever hospitals" in Egypt (1977)

	Patient's name (initials)	Virus isolation from specimens		
		Serum	Throat washing	Feces
1	A.I.M.	+	ND*	+
2	N.A.	+	ND	+
3	H.R.	+	--	+
4	M.F.A.	+	+	+
5	B.R.M.	+	+	-

*ND = Not done.

Table 6

Antibodies against RVF virus in sera of patients clinically diagnosed as RVF at the Sharqiya Governorate fever hospital, Egypt (1977)

No.	Name (initials)	Age (years)	HI antibodies		CF antibodies	
			Acute sera	Conv. sera**	Acute sera	Conv. sera**
1	B.M.B.	25	40*	2560	4*	64
2	M.A.A.	24	40	640	8	64
3	M.F.I.	26	20	2560	4	128
4	H.I.H.	29	40	160	8	128
5	S.A.R.	26	40	160	4	128
6	S.S.I.	28	20	80	4	64
7	S.F.I.	27	20	2560	4	64
8	R.M.A.	29	20	80	-	4

*Reciprocal of serum end point dilution.

**Acute and convalescent sera, 12 days apart.

Table 7

Isolation of Rift Valley Fever (RVF) virus from animals during the RVF epidemic in Egypt (1977)

Animal species	Locality	Date	No. tested	No. positive	Remarks
Sheep	Qalyubiya	7/11/77	14	3	Isolation from serum
Sheep	Sharqiya	8/11/77	2	1	Isolation from serum
Sheep	Giza	27/11/77	5	-	-
Sheep	Minya	17/12/77	22	17	Isolation from serum of 17 + liver of 2 dead sheep
Sheep	Sohag	24/12/77	5	1	Isolation from serum
Sheep	Giza	10/1/78	10	5	Isolation from serum
Cow	Giza	9/11/77	16	-	-
Cow	Giza	8/1/78	1	1	Isolation from serum
Camel	Aswan	29/11/77	30	1	Isolation from serum
Goat	Giza	9/11/77	1	-	-
Goat	Sharqiya	10/11/77	3	-	-
Goat	Giza	10/1/78	5	1	Isolation from serum
Horse	Cairo	1/12/77	18	1	Isolation from serum
Rodent	Sharqiya	29/10/77	3	-	-
Rodent	Sharqiya	30/10/77	3	1	Isolation from brain
Rodent	Sharqiya	31/10/77	2	-	-

Table 8

Sera from Egypt used for a retrospective serologic study for RVF, HI antibodies

Year	Number of sera from										
	Sheep	Buffalo	Cow	Camel	Pig	Donkey	Horse	Dog	Total animals	Human	Human & animals
1972	-	-	-	-	-	-	-	-	-	185	185
1974	463	-	-	-	162	-	-	-	625	682	1307
1975	522	130	120	75	120	-	-	-	967	940	1907
1976	569	170	146	90	104	49	15	40	1183	1376	2559
Total	1554	300	266	165	386	49	15	40	2775	3183	5958

Table 9

Serological study on animal sera from Egypt (1977-1978) for RVF virus HI antibodies

Animal species	No. tested	Positive sera No.	Positive sera %
Sheep	899	302	33.6
Camel	284	72	25.3
Buffalo	506	111	22.0
Cow	939	134	14.3
Goat	1222	68	5.6
Horse	361	18	5.0
Donkey	93	1	1.1
Total	4304	706	16.40

Table 10

Serological study on human sera from Egypt (1977-1978) for RVF virus HI antibodies

Locality	No. tested	Positive sera No.	Positive sera %
Lower Egypt	2102	359	17.1
Coastal governorates	339	11	3.2
Inland governorates	1763	348	19.7
Cairo	45	11	24.4
Upper Egypt	382	123	32.2
Egypt Total	2529	493	19.5

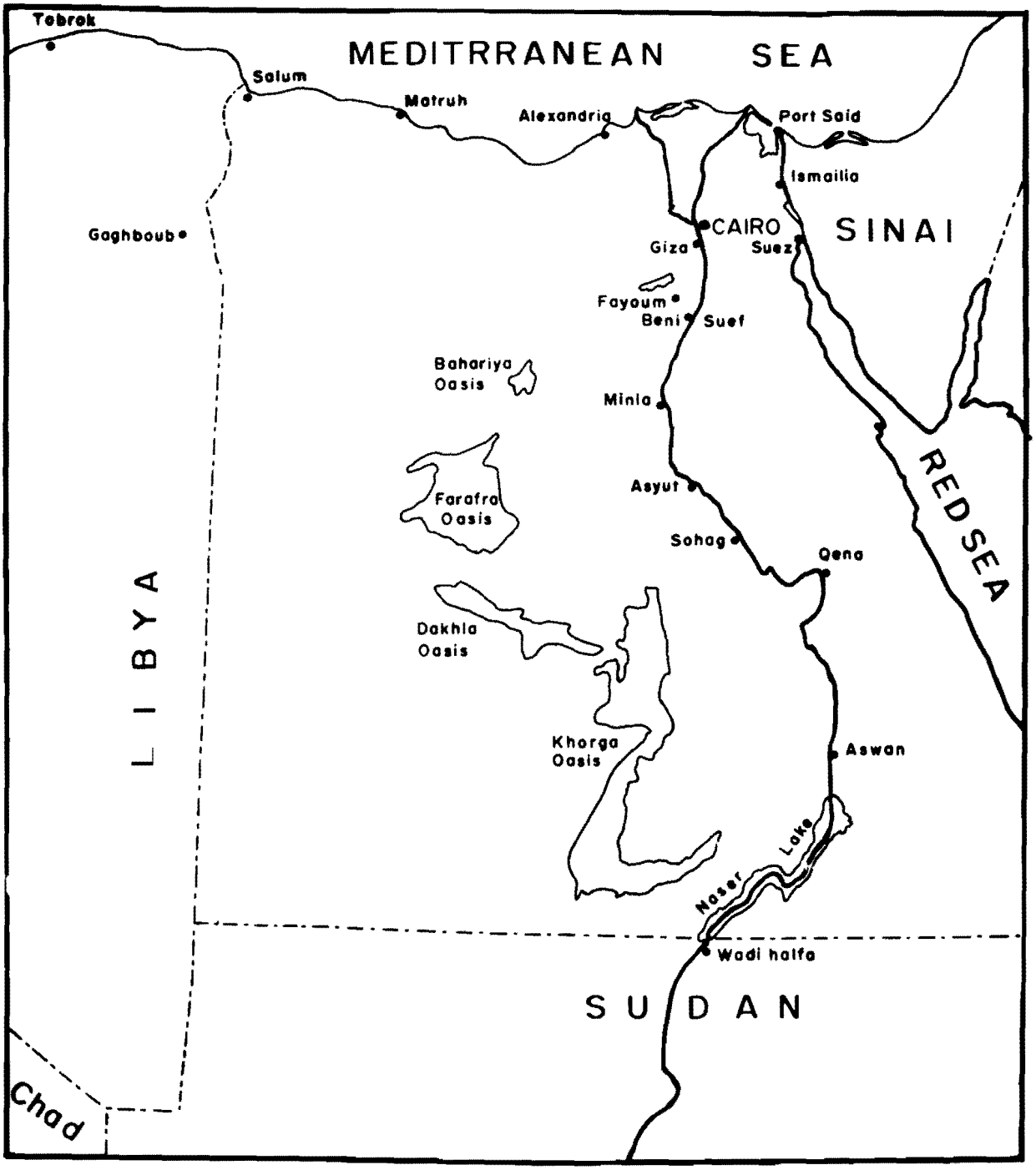
Table 11

Comparison of RVF and WN antibodies in different regions of Egypt

Locality	% positive sera	
	RVF*	WN**
Delta (coastal)	3.2	22.3
Delta (inland)	19.7	56.1
Upper Egypt	32.2	73.9
Egypt Total	19.5	50.3

RVF = Rift Valley fever

WN = West Nile fever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Salum
Matruh
Alexandria
Port Said
Ismailia
Gaghboub
CAIRO
SINAI
Giza
Suez
Fayoum
Beni Suef
Bahariya Oasis
Minia
Farafra Oasis
Asyut
Sohag
Qena
RED SEA
LIBYA
Dakhla Oasis
Khorga Oasis
Aswan
Nasser Lake
Wadi halfa
SUDAN
Chad

FIGURE 1.

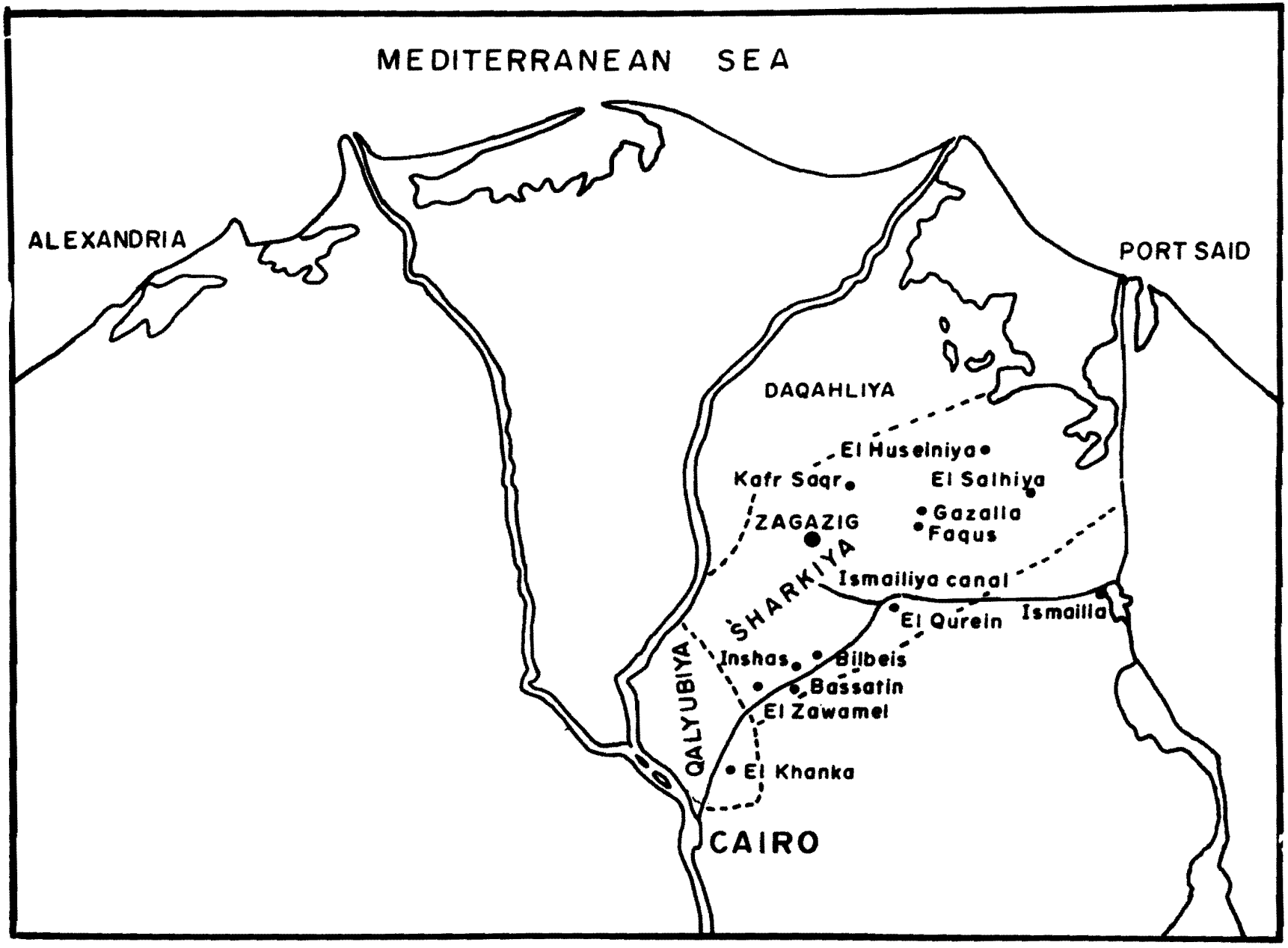

MEDITERRANEAN SEA
ALEXANDRIA
PORT SAID
DAQAHLIYA
El Huseiniya
Kafr Saqr
El Salhiya
ZAGAZIG
Gazalla
Faqus
SHARKIYA
Ismailiya canal
El Qurein
Ismailia
QALYUBIYA
Inshas
Bilbeis
Bassatin
El Zawamel
El Khanka
CAIRO

FIGURE 2.

REPORT FROM THE EGYPTIAN ORGANIZATION FOR
BIOLOGICAL AND VACCINE PRODUCTION
AGOUZA, EGYPT

ANNUAL REPORT ON RIFT VALLEY FEVER

Introduction

In October 1977, an outbreak of fever of short duration with severe constitutional symptoms, somestimes accompanied with jaundice and haemorrhagic manifestations took place in Sharkia Province. Later on other provinces were affected. Our laboratory received samples from human beings and animals from acute cases and convalescents for virus isolation and serological tests. This report will cover the period from October 1977 which is the start of the outbreak till December 1978 i.e. it will cover a period of 15 months. In some parts of the report will go back to periods preceeding this date in a trial to throw some light on the epidemiology of the disease.

Incidence

Patients admitted to fever hospitals and were diagnosed clinically as Rift Valley Fever and from whom samples were sent to our laboratory to confirm the diagnosis are taken as acute cases. In many occasions clinical sheets were sent with the samples.

The number of cases during this period was 665.

Seasonal Incidence

If we look to table 1 and graph 1 we can divide the disease into 3 periods :

1. The period from October to December 1977: The disease started in October with sudden onset of 156 cases, dropped slightly in November and then, suddenly to 39 cases in December.

2. The period from January to June 1978: The curve reached its minimum fluctuation between 3-10 cases with the exception of April when it was 21 cases.

3. The period from July to December 1978: The number of cases increased to 65 in July and remained within this limit till the end of November when it dropped again to 7 cases in December.

Geographical Distribution : (Table 2, graphs 2 and 3).

The disease appeared in 8 governorates as follows :

1. It first appeared in Sharkia in October and December 1977, stopped for 3 months and re-appeared in March 1978 and remained till the end of December to make a total of 435 cases.

2. In November 1977 cases were reported from Giza till January 1978 and then stopped completely till now. 90 cases were recorded in this period of 3 months.

3. By December 1977, three governorates were included which are Kalubia adjacent to Sharkia on the Eastern side of the Delta and two governorates in Upper Egypt which are Minia and Assiut. Whereas the disease appeared only for one month and was not recorded again from the last 2 Provinces, it also disappeared after November 1977 from Kalubia, but re-appeared in November and December 1978.

4. In April 1978 seven cases were discovered in Dakahlia on the North border of Sharkia and only for one month.

5. In November and December 1978, two new governorates were affected, Gharbia with 58 cases and Aswan in December with one case.

The causative agent was isolated from 5 of these 8 governorates (Table 5, graph 2).

Sex : (Table 1, graph 4)

The disease affects males more than females in a ratio nearly 5 males : 2 females.

Age Incidence : (Table 4, graph 5)

Out of all the cases, 251 had their age written in the sheets sent with their samples. Their age was classified in groups and it was found that the disease attacks any age probably the age from 15-45 is more susceptible.

Symptoms : (Table 3, graph 6)

213 sheets were sent containing the symptoms which were studied and were found that :

1. 169 patient had fever and 99 had constitutional symptoms. These symptoms were headache, rigors, pain in muscles and joints. Some cases suffer from hallucinations and even coma.

2. 142 cases i.e. about 67% of the total cases had haemorrhagic manifestations and patients had blood with their vomit, urine, stools or had epistaxis or subcutaneous haemorrhage.

3. Eye manifestations in 34 patients (16%) which were either congestion of the conjuctiva or loss of vision which was found to be due to macular degeneration.

Serological test

2537 human sera was collected from about 15 governorates in Egypt and tested serologically for R. V. antibodies. (Table 5 and graph 7). Nearly a positive result was obtained in all governorates. Some governorates show a high percentage of positivity because of the small number of sera collected from them as Giza, Cairo, Minia, Assiut and Kena, so their results do not represent their real situation. Such percentage is expected to drop very much if more representative samples are taken. But from the serological test we can conclude that a fair percentage of population have got antibodies in their sera and that Sharkia governorate which presents the highest percentage of cases and out of 1133 serum sample collected, the percentage of positive was 36.6%. Out of the total 2537 samples taken 526 were positive with a percentage of 20.7%.

Rift Valley Fever in animals

Serological Test

A. The period from November 1977 to December 1978 (Table 8, graph 7).

This is the period in which the human epidemic took place. 3774 blood samples of 8 species of animals in 14 governorates in Egypt were examined. The results as shown in the table was as follows :

Animal species	% Positive
Sheep	28.5
Cow	10.3
Buffalo	19.6
Goat	7.4
Camel	9.5
Donkey	4.0
Horse	6.3
Rodents	2.4

It means that antibodies were found in all species examined with variable percentage.

B. The period before November 1977 : (Table 9, graph 8).

1866 sera of 10 species of animals kept in the laboratory from 1973 were examined serologically and it was found that:

1. Positive results were obtained from sera of sheep, cow, and buffalo in February 1977 in Kena Province, then in 3 coastal governorates of Alexandria, Port Said and Matruh and in Aswan in the following months.

2. Sera of camel were positive in Cairo slaughter-house in March and April 1977 and in October in Aswan.

3. Sera of goat were positive in Helwan in Cairo and Matruh in April 1977.

4. No antibodies were found in other species which are horse, donkey, pig, mule and dogs.

Virus Isolation

520 samples from different species were examined. The virus was isolated from 35 samples. Tables 6 and 7 show the animal species, locality and date of isolation.

Abortions and Deaths

Data was obtained from Veterinary Section. Ministry of Agriculture and are represented in table 10 and graphs 9 and 10. This data covers the period from July 1977 to August 1978. It is noticed that :

1. The epidemic started in animals earlier than human beings by 5 months according to this data. It migh have started earlier than this data and passed un-noticed.

2. In 1977 the disease was present in 8 governorates :

2 in Lower Egypt viz Sharkia and Kalubia and 6 in Upper Egypt which are from South to North : Aswan, Kena, Suhag, Assiut, Minia and Giza.

3. In 1978 no cases were recorded from Giza and Suhag. On the other hand 3 new governorates were affected all in Lower Egypt which are Damietta, Dakahlia and Gharbia. Also in 1977 cases of abortions and deaths among sheep and cattle were quite distinct in 3 governorates :

(a) Aswan.

(b) Sharkia in Salhia in a farm of recently imported cows and in Diarb Nigm.

(c) Assiut in 2 farms one for Frizian cows in Beni Mor and the other for sheep in Hawatka.

4. In all mentioned governorates human cases appeared with the exception of Kena, Suhag and Damietta.

The Veterinary Section also made serological tests for different species of animals and they got results slightly higher than ours. Two isolates of virus were made by NAMRU 3. One from a sick cow in June 1978 from the Frizian farm in Beni Mor in Assiut and the other from a dead goat and its foetus in June 1978 from Minia.

Conclusion

In October 1977 an outbreak of acute febrile disease was reported first in Sharkia Province, later it extended to include 7 other governorates which are Kalubia on the Southern border of Sharkia, Dakahlia on its Northern border, Gharbia opposite to it on the other side of Damietta Branch of the Nile Delta and four governorates in Upper Egypt which are from North to South Giza, Minia, Assiut and Aswan. The first four governorates are close to each other, Minia and Assiut have common borders while Aswan lies in far South of Egypt on the Upper Border of Sudan.

The disease was characterised by sudden onset of fever of short duration of 2-5 days, headache, chills, rigors, muscle and joint pains, on many occasions jaundice, haemorrhagic manifestations in vomitus, stools, urine, cutaneous, peti-cheal rash all over the body and eye manifestation which include congestion of the conjunctiva and macular degeneration. Sometimes the disease ended fatally and the liver showed massive necrosis, fatty degeneration and peti-cheal haemorrhages at autopsy. Other tissues showed degenerative changes and peti-cheal haemorrhages specially the kidneys, heart and spleen.

The number of samples received from acute cases was 665, which is much less than the real number of cases reported from the Fever Hospitals in the affected governorates.

Males are more susceptible to the disease than females nearly in a ratio of 5:2 correspondingly. It affects any age. It was present all over the year but more in summer and autumn, the incidence being very low in winter and spring.

The virus was isolated from 64 cases, 50 of which were in Sharkia alone, the rest from Giza, Minia, Assiut and Gharbia.

HI test proved that 20.7% of population tested have antibodies in their blood.

As regards animals, it was noticed that an epizootic began in domestic animals in July 1977 which preceeded the epidemic in man by about 3 months. Within the period from July 1977 to August 1978 i.e. about one year it led to 958 deaths and 1126 abortions in these animals.

The following important points should be noted in this epizootic:

1. It first began in Aswan in July 1977 and remained restricted to Aswan in July and August.

2. From September 1977, it extended to the North in all governorates of Upper Egypt (with the exception of Beni Sweif) till it reached Giza then to 2 Provinces at the Eastern side of the Delta which are Kalubia and Sharkia.

3. In July 1978 it extended more to the North to include Dakahlia and Damietta, then to the West to Gharbia in the Middle of Delta.

4. The epizootic in camels caused 56 deaths and abortion of the camels. They were divided into 42 deaths and one abortion in Aswan and 14 abortions in Kena governorate which lies to the North to Aswan. The epizootic in camels was not found in any other governorate.

5. Human cases appeared in these governorates with the exception of Kena, Suhag and Damietta.

Serological test was done on 3774 sera of 8 species of animals in the period of human epidemic and antibodies were found in all species in a varying percentage. The test was also done on 1866 samples of sera kept in the laboratory since 1973.

Before the year 1977 all results were negative and the first sign of positivity was in February 1977 from Kena Province in sheep, cows and buffaloes, followed by camels in Cairo slaughter-house, then cattle in coastal provinces and camels in Aswan.

The virus was isolated from 35 samples from different species of animals during the epidemic. Out of these isolates, four were discovered in the mosquito culux pipiens : two from Zagazig in 1977 and two from Sama'ana both in Sharkia Province. These mosquitoes were caught alive, freshly fed and engorged with blood. If we add to this, the facts that mosquito is prevalent in Sharkia due to the presence of wide areas of breeding places in fields, gardens and water drains and that the epidemic nearly disappeared by the end of 1977 after the drastic anti-mosquito measures done, we can realise the role played by culux pipiens in the transmission of the disease.

Now remains the question how did the disease enter Egypt ? The following points may help in find an answer. It is known that the virus was first isolated in Kena in 1931. In 1963 neutralizing antibodies were demonstrated in human sera collected from several areas in Central African including Sudan. In 1973 an epizootic spread in Kosti District in Sudan from June to December. The morbidity was high reaching 100% among cattle. Humans were also invalued. Egypt import camels and sometimes cattle from Sudan.

They enter via Aswan where they are subjected to quarantine measures, but they also enter along the desert without control. Antibodies were first demonstrated in Kena in February 1977. The epizootic in Egypt began in July 1977 also starting in Kena, then spread to involve all Upper Egypt and the Eastern side of the Delta. Three months later, the human epidemic started among these localities.

Since the disease has no specific treatment, we hope that it will be controlled by the known anti-epidemic measures specially early diagnosis, notification and isolation of sick people and animals, quarantine measures and mosquito control. This necessitates the cooperation between the Public Health and Veterinary Authorities.

(Imam Z.E. Imam, Rifky El Karamany, Ferial Omar, Osman El Kafrawy and Medhat A. Darwish)

(Note from Editor: Tables 6-10 were not included with report.)

Table 1

Number of Acute Cases by Month and Sex in the period from October 1977 to December 1978

		No of cases	Males	Females
October	1977	156	131	25
November		122	91	31
December		39	20	19
January	1978	3	3	-
February		8	5	3
March		7	6	1
April		21*	9	5
May		3	2	1
June		10	6	4
July		65	43	22
August		58	40	18
September		65	48	17
October		32	23	9
November		69	45	24
December		7	5	2
Total		665	477	181

* 7 samples were sent without names

Table 2

Distribution of acute cases among Governorates

Month \ Governor.	Sharkia	Kalubia	Dakahlia	Gharbia	Giza	Minia	Assiut	Aswan
October 1977	93				47			
November	84	27			9			
December					31	7	1	1
January 1978					3			
February								
March	5							
April	14		7					
May	3							
June	8							
July	65							
August	55							
September	64							
October	32							
November	11	2		54				
December	1	1		4				
Total	435	30	7	58	90	7	1	1

In addition 36 cases of unknown locality

Table 3

Classification of Symptoms of 213 Patients as written in the sheets sent with their specimens

Symptom		No of patients
Fever		169
Constitutional symptoms		99
Jaundice		60
Hamorrhagic Manifestations :		
1. Hoemoptsis	31	
2. Subcut. hoemorrhage	42	
3. Epistaxis	15	
4. Meloena	42	
5. Hoematuria	12	
Total hoemorrhagic		142
Eye Manifestations :		
1. Congestion	19	
2. Loss of vision (Mac. Degen.)	15	
Total Eye		34

T a b l e 4

Age Groups of 251 Patients

Age Group	Number
- 10 years	15
- 20	61
- 30	50
- 40	59
- 50	37
Over 50	29
T o t a l	251

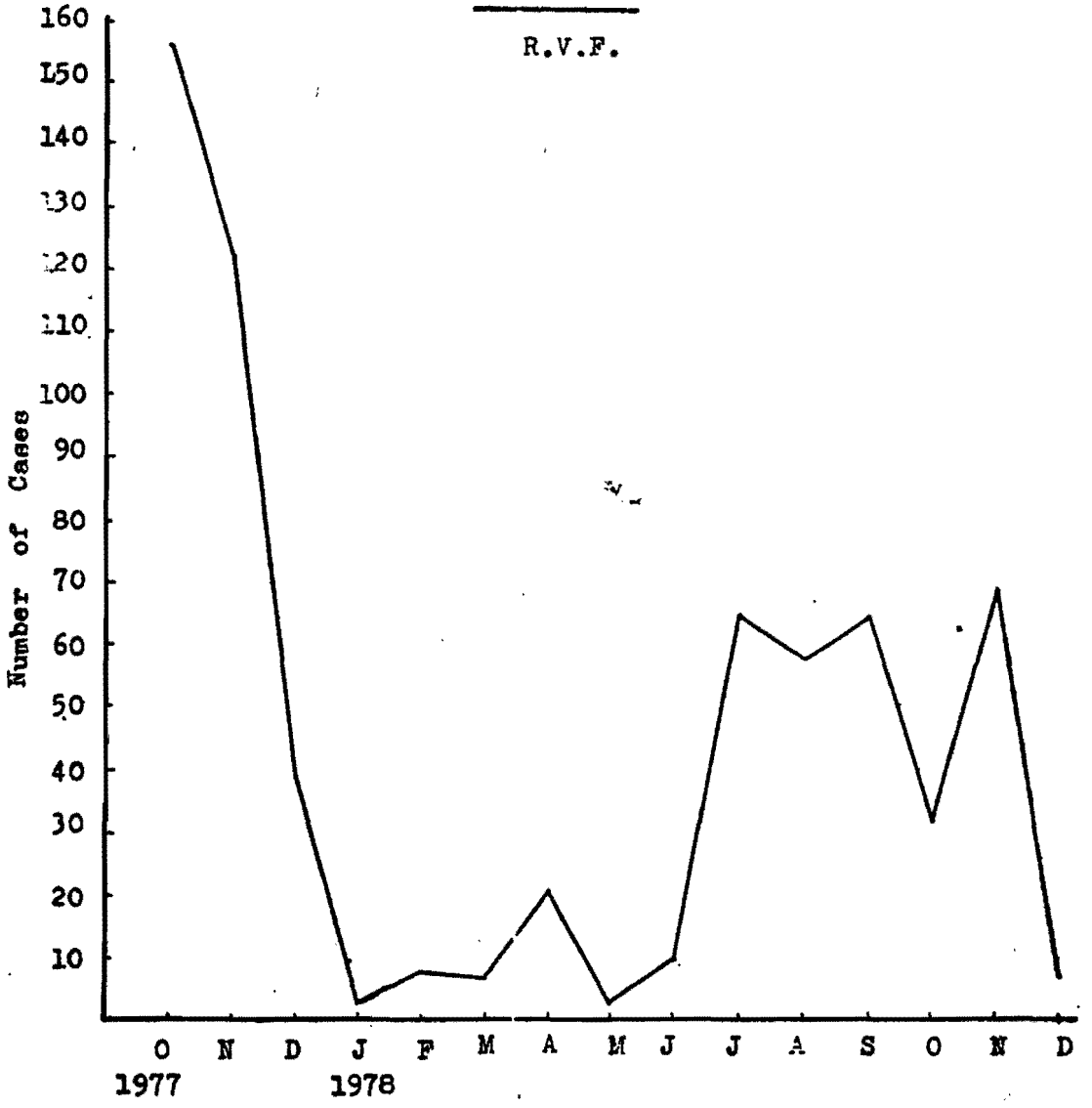
Table 5

Virus Isolation from Human Cases by Month and locality

	Sharkia					Giza		Minia	Assiut	Gharbia	Total
	Zagazig	Fakus	Hosania	Kafr Shukr	Abu Hammad	Pyramid	Imbaba				
October 1977	5					2	3				10
November	11										11
December								4	1		5
January 1978											
February											
March											
April											
May											
June	1	1									2
July		10	10								20
August		2	3	1							6
September											
October					1						1
November		2	1		1					4	8
December			1								1
Total	17	15	15	1	2	2	3	4	1	4	64

Graph 1

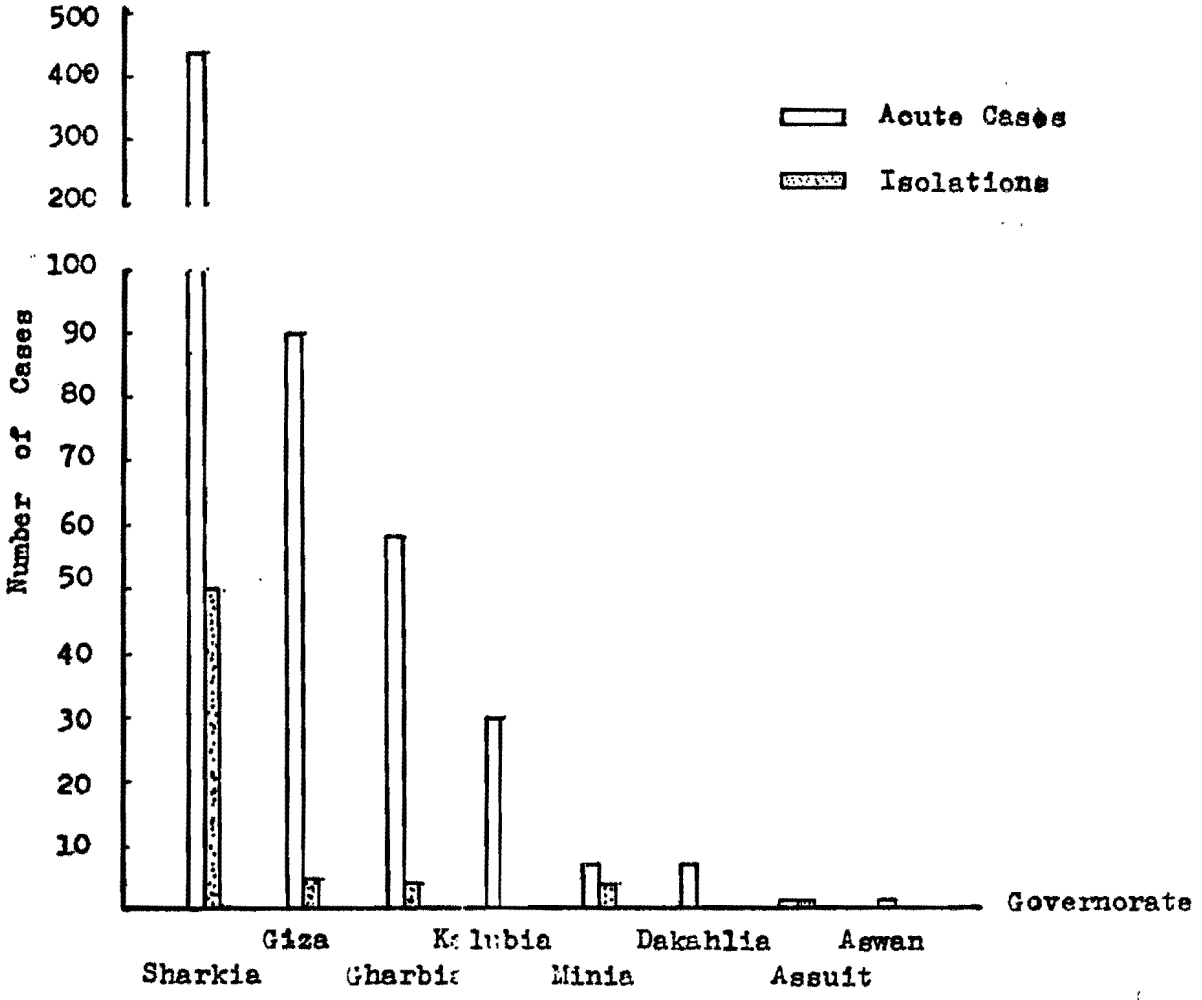
R.V.F.



Seasonal Incidence of Acute Cases of R.V.F.

Graph 2

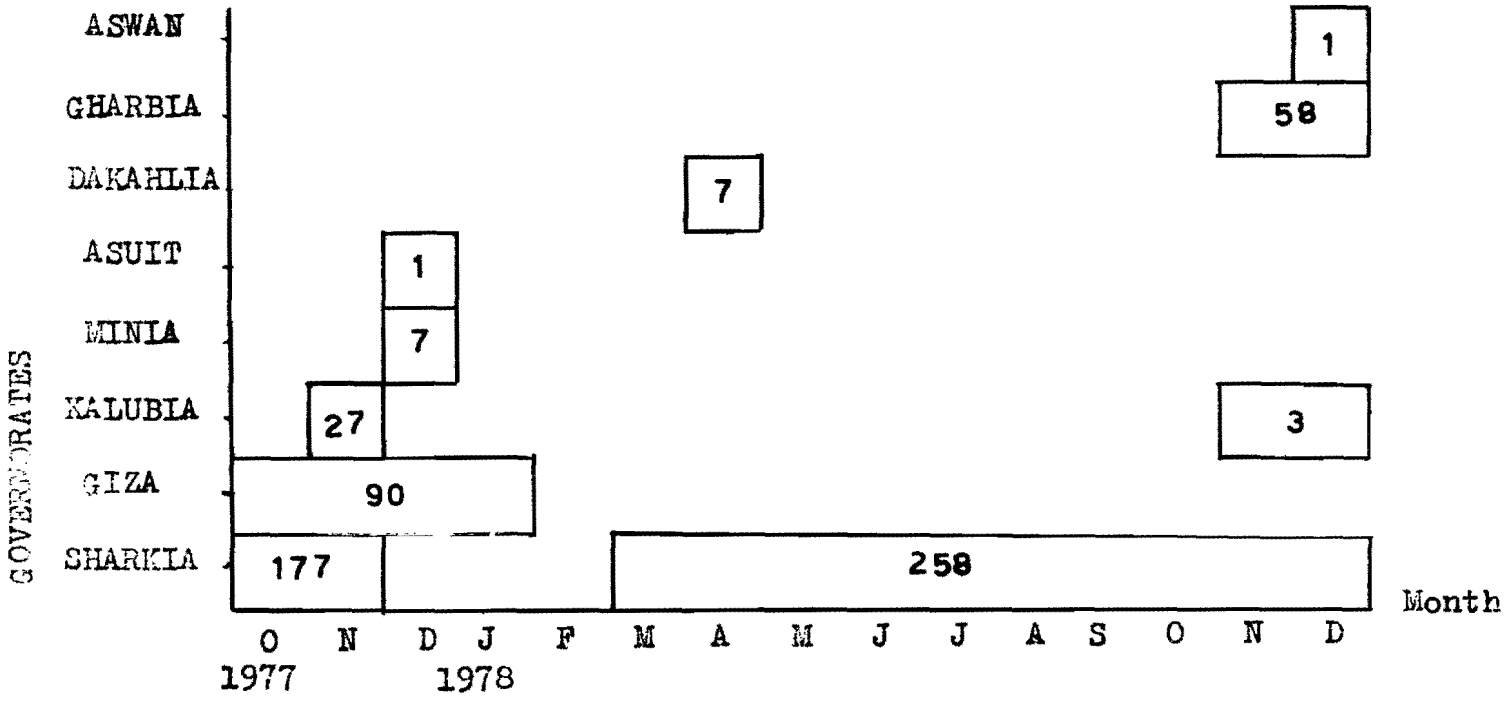
R.V.F.



Number of Acute Cases and Number of Isolations in different Governorates in the period from Oct.77 - Dec.78

Graph 3

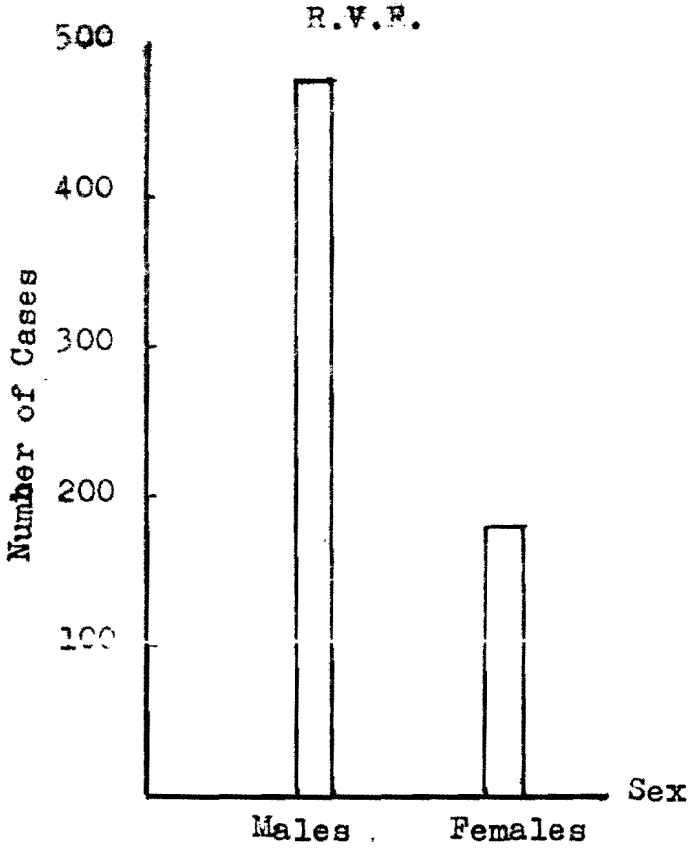
R.V.F



SEASONAL INCIDENCE AND NUMBER OF CASES IN DIFFERENCE GOVERNORATES IN THE PERIOD FROM OCT.77 - DEC.78. THE FIGURES INDICATE THE NUMBER OF CASES IN THE DRAWN PERIOD

Graph 4

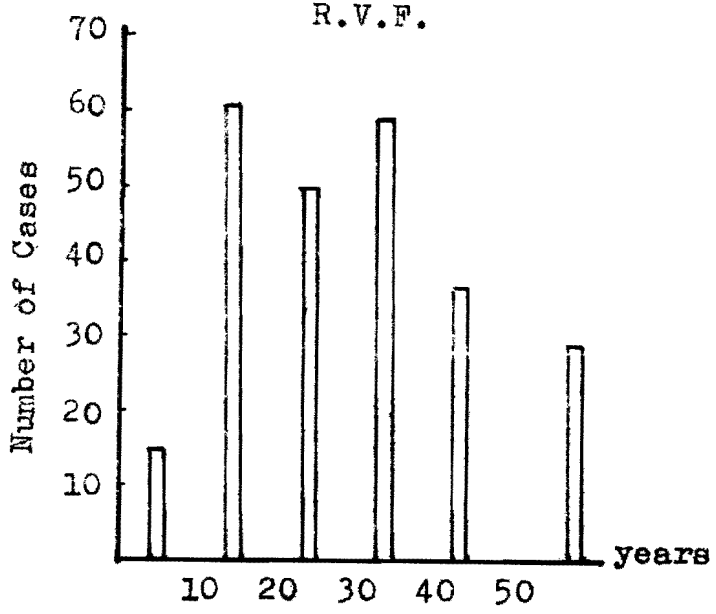
R.V.F.



Number of Males and Females

Graph 5

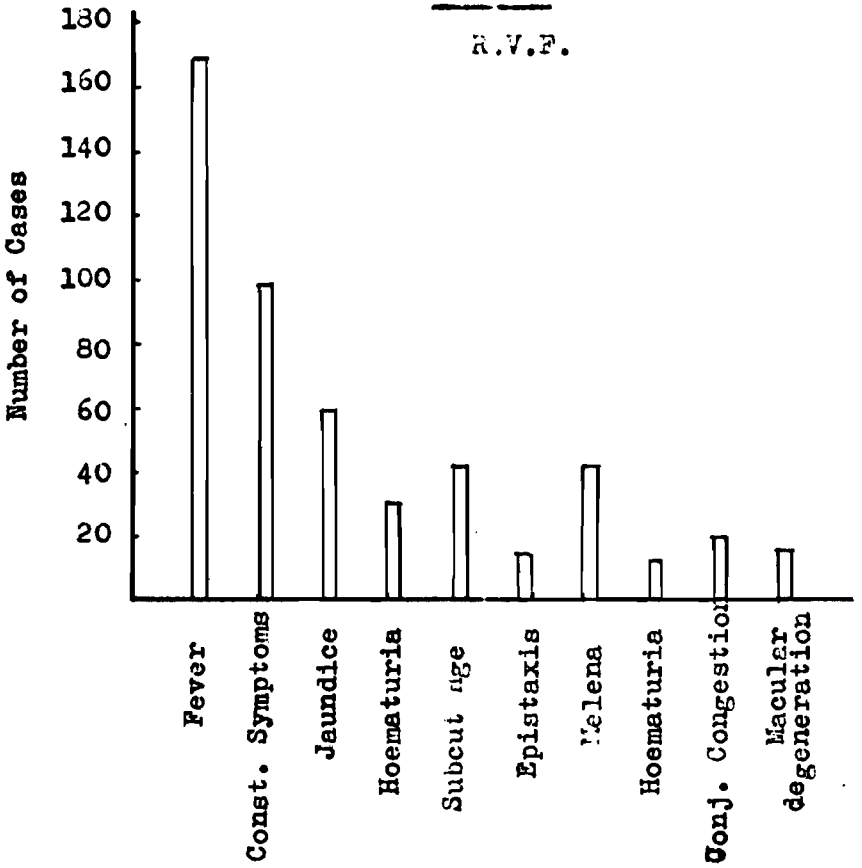
R.V.F.



Age Distribution of 251 Cases

Graph 6

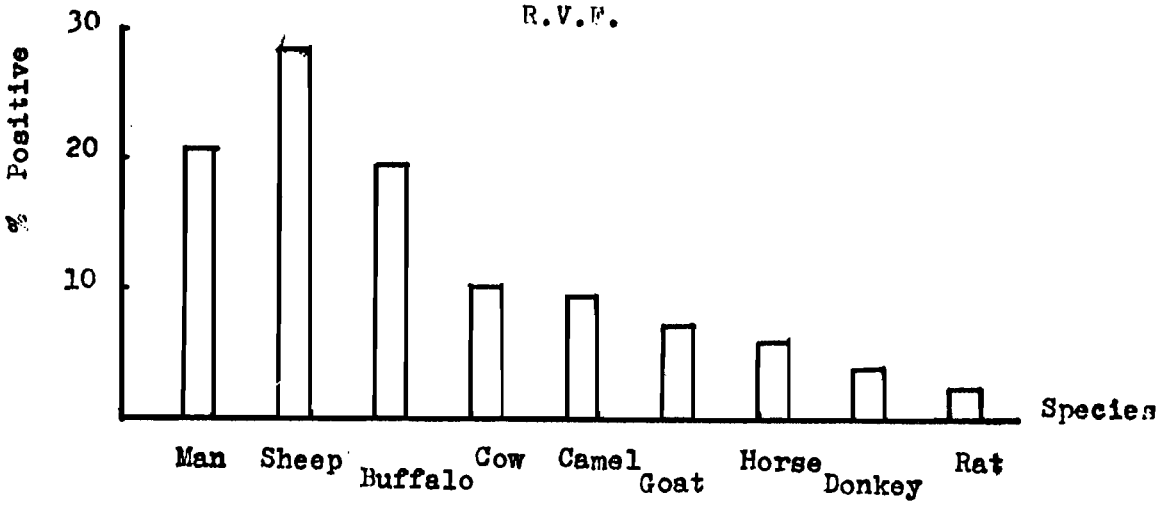
R.V.F.



Signs and Symptoms of 213 Patients

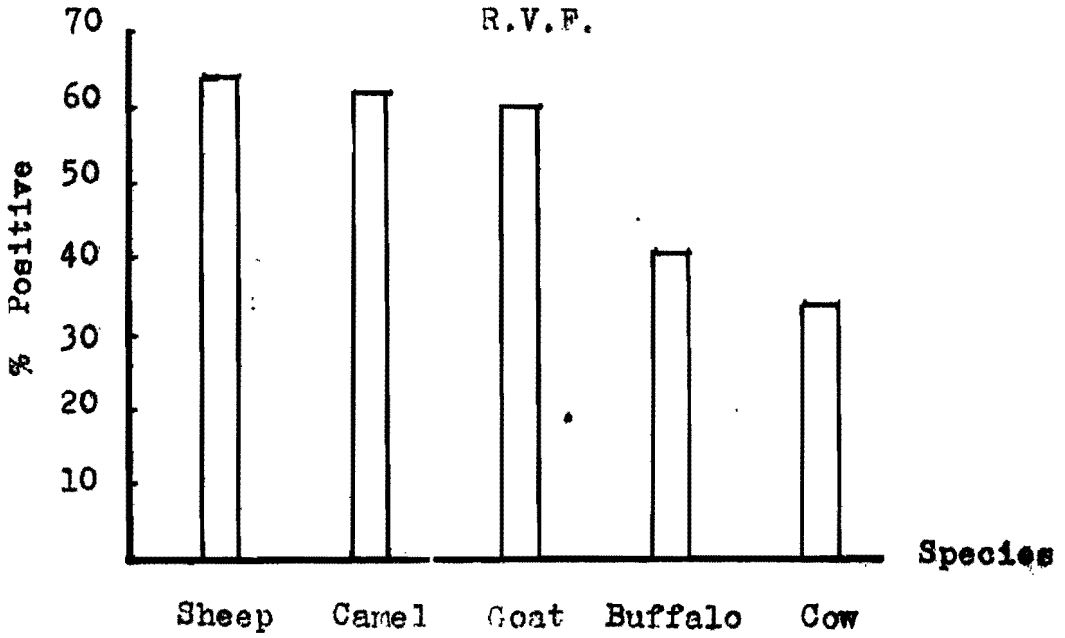
Graph 7

R.V.F.



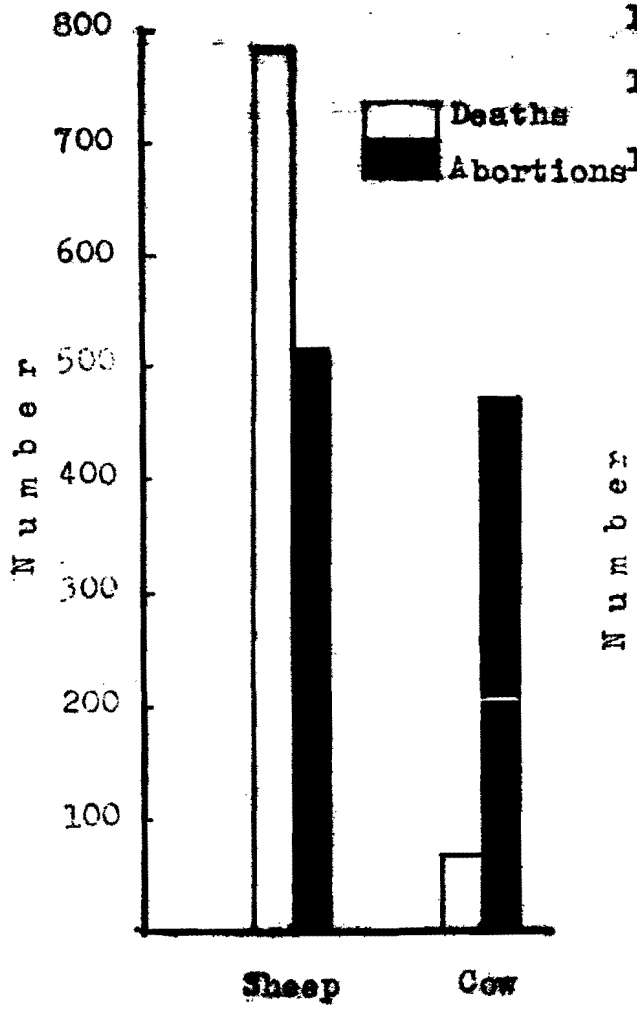
Serological Test on Human being and Animals in the period from Nov.77 - Dec.78.

Graph 8

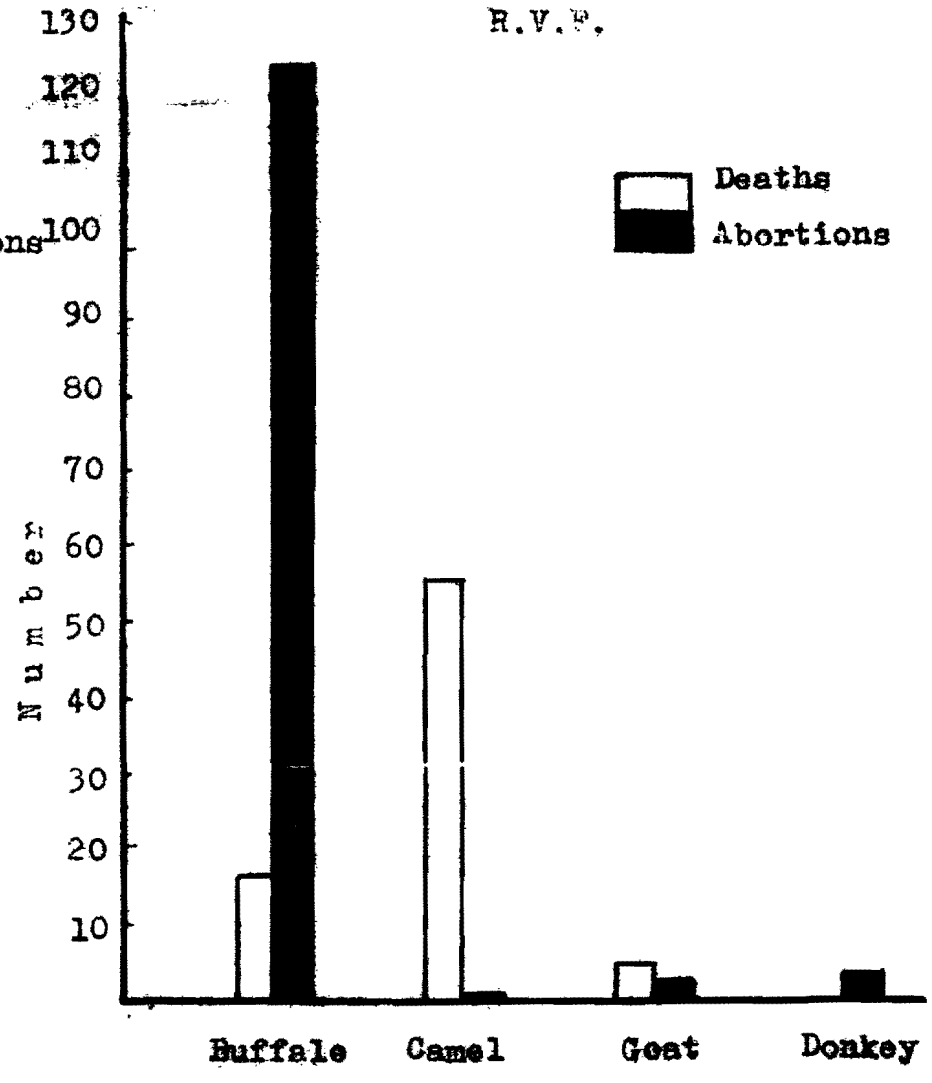


Serological Test on animals in 6 Governorates in the period from Feb.77 - Dec.77.

Graph 9



Graph 10



Deaths and Abortions in different species of Animals in the period from July 77 - August 78

REPORT FROM THE ARBOVIRUS LABORATORY
INSTITUT PASTEUR AND ORSTOM
Institut Pasteur - B.P. 220 - DAKAR (Senegal)

==========

During the second half of 1978, studies were continued out at our field station in Kedougou on the epizootic situation and to determine the aetiology and the dimensions of the recent outbreak in Casamance, near Gambian border.

1. VIROLOGICAL STUDIES

1.1 - Human blood samples

3 specimens collected from febrile ORSTOM technicians involved in arbovirus work in Kedougou area were inoculated into suckling mice without success.

1.2 - Wild vertebrate samples

82 blood samples collected from monkeys caught in Kedougou were inoculated into suckling mice (54 Erythrocebus patas, 8 Papio papio and 20 Cercopithecus aethiops).

Two strains of yellow fever virus were isolated from two young females Cercopithecus and perhaps 3 another strains of the same virus, yet in study, from Erythrocebus. These isolates, the first in Africa after these made by Andral in Ethiopia in 1968, indicate an important outbreak.

1.3 - Arthropods

Among mosquitoes caught during rainy season 1978 in Kedougou area, 22997 processed in 1042 pools were inoculated.

26 strains of Y.F virus were isolated and identified but 18 another, yet in identification, seem meanwhile to be Y.F strains. And as in 1977, Y.F is found in Aedes luteocephalus, in the beginning of the rainy season, and from october in Aedes gr furcifer taylori.

This year, no strain was recovered from male mosquitoes.

	A.luteocephalus	A.gr furcifer taylori	A.neoafricanus	A.vittatus
august	5			
september	2			
october	12	21	1	3

2. SEROLOGICAL STUDIES

2.1 - Human sera

2.1.1. From Bandia

63 sera from febrile children were examinated for arbovirus antibodies. 13 sera reacted with one or many group B antigens.

2.1.2. From Zaïre

Among 25 samples taken from soldiers of the Foreign Legion sent in mission in Kolwesi, one reacted with Y.F antigen at a low level in complement fixation test (CF).

2.1.3. From Upper Volta

448 sera collected in november 1977 and 378 in june 1978 were performed in HI test and showed many positive reactions with group B antigens. Flavivirus seem to be very active in this area.

2.2 - Wild vertebrate samples from Kedougou

Monkey sera caught in rainy season 1978, tested in Y.F CF revealed the similar pattern as in 1977. This antibody increase confirms the important Y.F outbreak if need be.

YF CF test in monkey sera collected in Kedougou

		N° tested sera	YF positive	
			N°	Percent
1977	sept.-october	23	0	0
	nov.-december	22	2	9%
1978	february	17	10	59%
	june-july	29	4	14%
	sept.-october	34	10	30%
	nov.-december	21	10	48%

3. INVESTIGATIONS IN CASAMANCE

In a population vaccinated against YF in april 1978, we have found among young children (1-15 years), one month after the outbreak of october-november 1978

3.1 A low YF virus circulation (few sera with isolated YF CF antibodies) but too raised to be only from vaccination origin.

3.2 A great and recent prevalence of Orungo virus (from 35 to 75% of sera by CF test).

3.3 8 sera among 29 collected (27,6%) in a single village, Touba M'Boyen ne, from children, were positive in immunofluorescence with Ebola virus.

All the sera were negative with Lassa and Marburg antigens.

Another investigations among the South Senegal population were performed to determine the Ebola prevalence.

Dr J.RENAUDET and Dr J.J. SALAÜN - Pasteur Institute
Dr M.GERMAIN and Dr J.L.CAMICAS - ORSTOM Dakar (Senegal)

FROM THE STAFF, VIRUS RESEARCH LABORATORY, IBADAN AND THE WORLD HEALTH ORGANIZATION ARBOVIRUS RESEARCH UNIT, ENUGU, NIGERIA.

Complement Fixing (CF) Antibodies to selected Arboviruses in Nigeria.

Human sera collected from different parts of Nigeria were tested in CF tests for antibodies to six arboviruses in Nigeria: Chikungunya (CHIK), Orungo (ORU), Yellow Fever (YF), West Nile (WN), Dengue-2 (DEN-2) and Potiskum (POT).

The results of the studies are shown in Table 1. There is almost an identical pattern in the antibody picture for both CHIK and ORU in Ibadan, where we have evidence for a five year epidemic cycle of CHIK virus infection among children below 5 years of age.[1,2] The incidence of CF antibodies to CHIK virus in these studies tend to support this speculation. We suspect, however, the high incidence to CHIK virus in the above 40 years age group, may be due to an exposure to other related group A viruses. With ORU virus, the situation is slightly different. In a previous study[3], CF antibodies to ORU virus were found to persist longer than N antibodies in monkeys experimentally infected with ORU virus. If the response in man is similar to that of monkeys, the CF antibodies we found in these studies may be from a long past rather than a recent infection. Further studies are still going on with these sera. There is evidence for considerable group B virus activity throughout the country.

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1. Moore, et. al. (1974): An. Trop. Med. Parasit., 68: 59.

2. Tomori, et. al. (1975): Trop. Geogr. Med. 27: 413.

3. Tomori, et. al. (1978): Trans. Roy. Soc. Trop. Med. Hyg., 72: 230.

Immunological reactions of Rift Valley Fever virus Strains from East and and West Africa.

Three strains of Rift Valley fever virus, namely Nigerian (NIG), Smithburn's neurotropic (SNT), and Lunyo variant (LUN) were compared by complement fixation (CF), neutralisation (N), haemagglutination/haemagglutination-inhibition (HA/HI) and agar gel diffusion (AGD) tests. They showed reciprocal cross reactivity in CF tests, (Table 2). In N tests, using immune sheep sera, there was reciprocal cross-neutralisation between the NIG and SNT strains, but not with the LUN strain, the antiserum of which neutralised both NIG and SNT antigens whereas the reverse was not the case (Table 3). When hyperimmune mouse ascitic fluid was employed in N tests, there was cross-reactivity between the three strains. Both the NIG and SNT strains yielded haemagglutinins, (Table 4), but not the LUN strain. Furthermore, by the antibody absorption and AGD techniques, the NIG and SNT strains were found to be identical and distinct from the LUN variant strain. The techniques found most useful in distinguishing between the three strains were HA and AGD. Laboratory neuro-adaptation of the classical pantropic virus did not appear to affect its haemagglutination activity.

(O. Tomori)

TABLE 1

COMPLEMENT FIXING ANTIBODIES TO SELECTED ARBOVIRUSES IN HUMAN SERA COLLECTED FROM DIFFERENT PARTS OF NIGERIA.

AGE RANGE IN YEARS	TOTAL NUMBER TESTED	NUMBER POSITIVE* TO						
		CHIK	YF	WN	D2	POT	TOTAL GROUP B	ORUNGO
0 - 10	42	25	1	0	0	1	2	24
11 - 20	418	151	20	14	6	0	20	134
21 - 30	540	229	29	15	7	5	56	216
31 - 40	133	49	7	4	6	3	20	55
40 and above	45	23	3	3	0	1	7	21
	1178	450	60	36	19	10	125	477

*serum dilution of 1:8 or above giving at least a 3+ fixation with 1:8 dilution of antigen was regarded as positive.

Table 2

Cross-Complement fixation tests with RVFV strains, immune sheep sera and immune mouse ascitic fluids.

Antibody		Antigen* NIG	SNT	LUN
Immune sheep sera	NIG	128+	64	64
	SNT	8	8	16
	LUN	32	32	64
Immune mouse ascitic fluids	NIG	32	64	32
	SNT	32	64	32
	LUN	64	64	32
Hyperimmune mouse ascitic fluids	NIG	1024	128	512
	SNT	1024	128	512
	LUN	2048	256	512

* 8 units of antigen used

+ Reciprocal of highest positive serum dilution.

Table 3

Cross-Neutralisation tests with RVFV strains, immune sheep sera and hyperimmune mouse ascitic fluids.

Antibody		Antigen* NIG	SNT	LUN
Immune sheep sera	NIG	2.3*	0.5	0.0
	SNT	2.1	1.5	0.0
	LUN	3.0	1.7	1.4
Hyperimmune mouse ascitic fluids	NIG	3.1	2.0	1.6
	SNT	1.5	1.5	1.7
	LUN	2.9	1.2	1.7

* Neutralisation index, expressed index

Table 4

Haemagglutination titres of RVFV strains at 37°C and at varying pH values.

pH Values	NIG GRBC*	NIG CRBC*	SNT GRBC	SNT CRBC	LUN GRBC	LUN CRBC
5.8	0+	0	0	0	0	0
6.0	80‡	160	160	160	0	0
6.2	160	160	160	40	0	0
6.4	40	40	20	0	0	0
6.6	20	0	0	0	0	0
6.8	0	0	0	0	0	0

* GRBC = Goose red blood cells, CRBC = Chick red blood cells

\+ 0 = 10

‡ Reciprocal of HA titre

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

This report summarizes the results of our arbovirus laboratory for the year 1978. During this year ongoing virological and serological studies were actively maintained to collect more information on the life cycle of sylvatic yellow fever at our field station. This station was established at Bozo, 110 km north of Bangui, during the year 1973.

1 - Virological studies.

1.1. Human blood samples.

86 blood samples were inoculated into suckling mice. One strain of Chikungunya was isolated.

1.2. Arthropods.

Mosquitoes : 42,141 mosquitoes collected at the station were divided into 1,711 pools and inoculated into suckling mice :

6 strains of yellow fever virus, 28 strains of Chikungunya virus and 6 strains of Orungo virus were isolated from *Aedes africanus*.

From other mosquitoes collected at Bozo during 1978 have been isolated also : 1 strain of Bunyamwera virus from *Anopheles gambiae* and 1 strain of Bwamba virus from *Aedes fowleri*.

If the identification of these yellow fever isolates is confirmed by the WHO Reference Centre in Dakar, it will

be the third year (1974,1977, 1978) in which this virus has been isolated from mosquitoes during the rainy season (september-november) at Bozo (Central African Empire).

Ticks : from 1,201 ticks collected at Bangui and divided into 63 pools, the following were recovered : 8 strains of Dugbe virus isolated from Amblyomma variegatum.

1.3. Wild vertebrates.

Of 74 blood samples from birds in the Bangui area which were inoculated into suckling mice, no isolates were obtained.

2 - Serological studies.

82 serum samples collected after the 1977 rainy season (january-march) from children under 10 years old in the Bozo area were studied with IHA and CF test for group A, group B and Bunyamwera viruses. From these, 1 serological conversion for the yellow fever virus and 1 serological conversion for Bouboui virus were found.

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REPORT FROM THE UGANDA* VIRUS RESEARCH INSTITUTE
ENTEBBE, UGANDA

*Formerly the East African Virus Research Institute.

An Epidemic of CHIK in Entebbe

Four strains of CHIK were isolated from four Entebbe residents who reported to the Institute Clinic between the 14th-19th January 1978 with almost similar complaints: fever, headache, backache, myalgia and joint pains. They were examined, had blood samples taken and then treated for bacterial infections and malaria. Sera from their blood samples were inoculated into newborn mice which became sick and died 2-3 days later. These isolates at 1-6 mouse passages conducted so far, kill suckling mice inoculated by IC and IP routes and also adult mice injected by IC but not those inoculated by IP. They are all ether sensitive and pass through Seitz filters. Reisolation trials have been successful in each case.

Five other strains of CHIK, three from man and two from mosquitoes were also made. The human isolates were obtained on the 1st February and 1st March from residents of Entebbe. The last human strain was acquired on the 1st June from a resident of Buwaya, 6 km from Entebbe. Mosquito strains were isolated from Aedes africanus collected from Zika Forest (8km from Entebbe) on the 15th May and 1st June. All these strains have similar properties described above for the 4 isolates of CHIK. They have also been reisolated from original specimens.

Isolation of 4 strains of CHIK in the same week and then two other strains of the same virus in the next 1½ months from sick people living in the same area indicates that there was an epidemic of CHIK in Entebbe village. This epidemic reached a peak in January and then tapped off in the next two months. Perhaps more isolates would have been made if specimens were in addition received from patients visiting hospitals in Entebbe with suspected viral infections. All the donors of these isolates, had febrile illnesses indicating the importance of CHIK in human health.

Although CHIK is transmitted by mosquitoes, particularly Ae. africanus, none of the mosquitoes collected during regular and special catches from various localities in Entebbe yielded this or other viruses at the time of the epidemic. However, 5 months after detecting CHIK in Entebbe two strains of this virus were isolated from Aedes africanus caught at the tower of Zika Forest. It is not known whether the two isolates from Zika Forest originated in Entebbe or were from a local infection. Similarly the origin of the isolate from Buwaya village was not determined.

Isolation of Congo Virus from Human Blood

A young boy, 12 years of age, from Nakasozi village visited the Institute Clinic on the 11th May 1978 with complaints of fever, headache, nausea and vomiting. On examination his temperature (38.4°C) was found elevated. A sample of his venous blood was withdrawn for attempted virus isolation. He was then given symptomatic treatment and then sent home. He did not return to the clinic.

Serum from his blood was inoculated into newborn mice. On day 9 p.i. mice became sick with symptoms of paralysis of hind legs and others died. Brain tissues from some of the sick mice were passaged into groups of other suckling mice. On passage 2 this virus gave titres of 6.5 log/0.02 ml in NB mice injected either by IC or IP routes; adult mice inoculated by these routes did not become sick. It is ether sensitive and passes readily in Seitz filters. Reisolation test was positive.

After failing to detect HA with fluorocarbon-extracted antigen followed by protamine treatment CF test was used to screen this virus against 30 group antisera and 30 monotypic antisera. The results related it to Congo virus. Cross CF test showed that this isolate is a strain of Congo virus and neutralization test conducted in mice confirmed these findings as shown below:-

Cross CF and Neutralization Tests Between Congo Virus and Isolate SG 39281

ANTIGEN	ANTISERUM			
	Congo		SG 39281	
	CF	NT	CF	NT
Congo*	128	4.1	64	1.8
SG 39281	256	0.8	256	1.2

* Strain V 3010 was used in CF test and strain Ib 10200 was employed in NT

Isolation of Zika Virus from Mosquitoes and Man

A pool of 4 Ae. africanus mosquitoes collected on the 23rd October 1978 from Zika Forest was inoculated into suckling mice two days later. Inoculated mice became sick on day 8 PI and died 1-3 days later. Suspensions of brains from some of the sick mice were prepared and then inoculated into both NB and adult mice. The agent titred over 8.5 log/0.02 ml by both IC and IP routes in suckling mice and it was pathogenic for adult mice inoculated by IC route but non-pathogenic for adult mice inoculated intraperiteneally. It is ether sensitive and passes readily through Seitz filters. It was reisolated from the original mosquito suspension.

Fluorocarbon-extracted antigen of this virus gave HA titre of 1:320 at an optimum pH 6.2 and this was used in HI tests which related it to ZIKA. Neutralization tests are underway to confirm this finding.

Twenty nine days (23.11.78) after mice were inoculated with mosquito suspension which yielded this virus, an animal attendant who feeds experimental animals and cleans their cages reported to the Institute Clinic with a febrile illness accompanied by headache, myalgia and joint pains. His temperature was 38.2°C. He was bled for virus isolation and then given antibiotics and chloroquine. He recovered without complications in 3 days after which he resumed duty.

Serum from his blood was inoculated into NB mice. On day 4 PI one of these mice looked suspeciously sick. Its brain was therefore collected and its suspension was given into two litters of suckling mice. These became sick on day 7 PI and were all dead two days later. This agent is pathogenic for adult mice inoculated intracerebrally. Their illness lasts for 3-6 days before they die or in rare cases recover . Adult mice inoculated intraperitoneally remain normal. This virus is ether sensitive and passes through Seitz filters. It has been identified by HI test as a strain of ZIKA and has been shown to be indistinguishable from the mosquito isolate. It was reisolated from the original serum. Sera taken at intervals from this worker have not yet been tested for seroconversion.

The source of this human isolate is most likely to be experimental animals infected with the mosquito isolate described above because ZIKA was not handled in the laboratory until the 25th October when mice were injected with mosquito suspension that yielded isolate MP 15738. Since then this agent was used extensively in mice for the preparation of antigens from infected brains and for the passage studies. It is of interest to note that this was the second laboratory infection of Zika virus in this Institute. The first was reported in 1964 and it occurred in a laboratory worker who had visited Zika Forest and was bitten by mosquitoes 23 days before he became ill (Trans. R. Soc. Trop. Med. & Hyg. 58: 335). His, like this case, was a mild febrile illness of short duration.

HI Antibodies to Nine Arboviruses in Human Sera from Various Places in Uganda

Sera kept in storage since 1961 are scheduled to be screened against at least 9 arboviruses known to infect man. This study is being conducted to determine the distribution of these viruses in East Africa in man and other species of animals. These sera are scheduled to be screened also against a new virus (Z 52969) isolated in 1977 from bat blood in Kasokero cave (Masaka District) to determine its distribution and to find out whether or not it is newly introduced.

This study started with human sera of 1961 from all over Uganda being tested by HI against 9 viruses. The results obtained so far on 180 sera indicated that apart from CHIK in West Nile these viruses had a low prevalence all over Uganda (Table 1). CHIK was quite prevalent in West Nile with 81.8% positive in that District and with 41.1% in the country.

Detection of antibodies to the new isolate in Karamoja and Teso districts suggests that this virus is well spread in Uganda and has been in the country at least since 1961.

(M. Kalunda, L.G. Mukwaya, S.D.K. Sempala, M. Lule, E. Sekyalo, Y. Senkubuge, C. Mawejje, A. Mukuye and J. Muyinge, Uganda Virus Research Institute, P.O. Box 49, Entebbe, Uganda)

Table 1: HI Antibodies to Arboviruses in Human Sera from Various Places in Uganda

DISTRICT	No. Tested	Z 52969	CHIK	WSL	NTAYA	BUN	YF	WN	KADAM	WET
Acholi	9	-	7	2	1	-	1	-	-	-
Bukedi	2	-	1	1	1	-	1	-	-	-
Bugerere	1	-	-	-	-	-	-	-	-	-
Bugisu	2	-	-	-	-	-	-	-	-	-
Karamoja	83	2	5	1	1	-	2	1	1	1
Kampala	1	-	-	-	-	1	-	-	-	-
Lango	1	-	1	-	-	-	-	-	-	1
Mubende	1	-	-	-	-	-	-	-	-	-
Teso	11	1	4	2	1	-	1	-	-	2
Sudan	2	-	1	-	-	-	-	-	-	-
West Nile	66	-	54	-	-	2	-	-	-	3
Entebbe	1	-	1	-	-	-	1	-	-	-
Total	189	3	74	6	4	3	6	1	1	7
% Pos.		1.7	41.1	3.3	2.2	1.7	3.3	1.1	1.1	3.8

REPORT FROM THE VETERINARY RESEARCH LABORATORY
SALISBURY, RHODESIA

1. Rift Valley fever (RVF)

It was expected, in the wake of the 1978 RVF epizootic, that the disease would flare up again during the current wet season, possibly in January. However, the rains have been sparse so far and the expected outbreak has not materialised by mid-February, although isolated serodiagnoses have been made in enzootic areas.

Reports on human RVF deaths (Swanepoel et al., 1979) and an encephalitis case (Maar et al., 1979), have now been published.

2. African horsesickness (AHS)

Over the years there have been instances of vaccine apparently failing to afford horses protection against AHS. There are nine known serotypes of the virus and a tenth has been reported from Kenya (Davies, 1976). Vaccine in use here contains attenuated strains of types 1 to 6. Isolates obtained in Rhodesia during recent years have been identified as belonging to types 5, 6 and 7, but two identical isolates have so far failed to cross-react in neutralisation (NT) tests with types 1 to 9 and may prove to be related to the Kenya virus, or else constitute yet another serotype.

Riding school horses which had been vaccinated an average of eight times had moderate to high NT titres against the full range of serotypes 1 to 9 of the virus. A group of horses which lacked antibody, acquired serological immunity of increasing titre and broadening antigenic range when vaccinated four times at six-monthly intervals. Race horses which had been vaccinated once only as foals, lacked antibody altogether or had low titres to two or three serotypes of virus. Repeated re-vaccination appears to be beneficial and there seems to be no sound basis for the practice of not vaccinating race horses, particularly since the increasing immunity resulting from repeated vaccination makes it unlikely that horses will react to vaccine in such a way as to interfere with training. Isolation of AHS virus from the organs of two young foals, suggests that there may be need to modify the current practice of delaying initial vaccination until the age of six to nine months to allow maternal immunity to wane.

3. Equine abortion

In 1974 there was a dramatic increase in the number of abortions which occurred in Thoroughbred studs and over the next few years abortion rates ranged from 12 to 100 per cent in various parts of the country. Equid herpesvirus I (EHI or Equine rhinopneumonitis) was isolated from the only aborted foal which showed histopathological lesions characteristic of infection with this virus, and it is concluded that EHI has not been a major cause of abortion in this country.

Isolation of RVF virus from two aborted horse foetuses is discounted since the specimens may have become contaminated from infected cattle foetus specimens submitted in the same parcel. No conclusions can yet be drawn on the significance of the isolation of Mazoe virus from two aborted horse foetuses. Mazoe virus was isolated originally from multimammate mice (Swanepoel et al., 1978) and is as yet incompletely characterised.

Unpublished information from South Africa indicated that the mosquito-associated virus, Middelburg (MID), may be related to a hepatitis syndrome and abortion in horses. HAI titres to MID virus of up to 1:1280 were demonstrated in the sera of horses and three aborted foetuses from various parts of Rhodesia, and it is possible that the virus may be associated with a proportion of the abortions seen in Rhodesia. Neutralising (NT) antibodies to a strain of equine encephalosis (EE) virus (isolated from aborted horse foetuses in South Africa and thought to be associated with midges) were also fairly common in the sera of horses in Rhodesia and occurred in the sera of two aborted foetuses. However, retrospective tests show that NT antibodies to a non arthropod-borne togavirus, equine arteritis (EA), first appeared in Rhodesia at the onset of the serious abortion problem in 1974 and subsequently became widely distributed. High antibody titres occurred in 24 sera from aborted foetuses. Cell cultures employed in the original testing of specimens had not been suitable for the isolation of EA virus and retrospective testing in monkey kidney cell cultures of specimens stored at -20°C, failed to produce isolates.

4. Other arboviruses

A Simbu group agent isolated from the brain of a horse which died of suspected rabies, has been subjected to cross-CF tests with Simbu, Shuni and Akabane viruses. There was complete cross-reaction with Shuni virus, but not with the other two viruses. Cross-NT tests have still to be done.

Two unidentified viruses isolated from aborted cattle foetuses during the 1978 epizootic, may be alphaviruses.

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REPORT FROM THE EVANDRO CHAGAS INSTITUTE, FSESP, BRAZILIAN . MINISTRY OF HEALTH, BELÉM, BRAZIL
AND THE
UNITED STATES ARMY MEDICAL RESEARCH UNIT-BELÉM APO MIAMI 34030

SIMULTANEOUS INFECTIONS OF ST. LOUIS ENCEPHALITIS VIRUS AND *LEPTOSPIRA* IN A PATIENT FROM BELÉM, PARÁ, BRAZIL, 1978

As part of a viral hepatitis surveillance program which is carried out in Belém, Pará, Brazil, a jaundice patient was seen on October 6, 1978 . The patient (BEL 13871), a 21 year old female, became sick on September 29 with fever, chills, severe headache, abdominal pain, myalgia, arthralgia and later with jaundice, and was hospitalized on 4 October . Physical examination of the abdomen on admission revealed disseminated tenderness to palpation, especially in the epigastric and right hypochondrial regions . Laboratory studies revealed a bilirubin level of 6.0 mg/100 ml of serum in a sample drawn on day 8 of illness . Levels of SGOT, SGTP and blood urea were also moderately elevated . White blood cell counts performed four times during the course of her illness were each within normal limits . Urine analysis revealed a 3+ albuminuria, 30 neutrophils per field, rare red blood cells and casts . A blood sample collected on 6 October, the 8 th day of illness, yielded a strain of St. Louis encephalitis (SLE) virus when assayed in infant mice . The clinical manifestations gradually subside, and the patient was discharged free of symptoms after being hospitalized for a total of 16 days . With the exception of severe headache, no signs of central nervous system involvement were observed during the period of her hospitalization .

Besides the isolation of SLE virus, a concurrent *Leptospira* infection was diagnosed by seroconversion using a macroscopic agglutination test and pools of *Leptospira* antigens prepared by Difco Laboratories+ . The patient's serum collected on day 8 of illness showed no reaction to any *Leptospira* pool tested ; however, serum collected on day 18 of illness showed a 2+ agglutination with pool 3 (containing antigens to *L. autumnalis*, *L. pomona* and *L. wolffii*) . Agar gel diffusion and counterimmunoelectrophoresis tests on a serum sample collected on day 8 of illness were negative for HB_s Ag, and no malaria parasites could be detected in a blood smear taken on the same day .

+ Difco Laboratories, Detroit, MI, USA

The virus isolated from this patient, BeH 355964, was identified as a strain of SLE virus by complement fixation (CF) and neutralization (NT) tests . Results of CF and NT using the isolate and various Flaviviruses known to occur in Brazil and their homologous mouse hyperimmune ascitic fluids are shown in Table 1 . It is apparent from these results that the isolate is closely related to the Belém prototype of SLE virus, but distinct from the other Flaviviruses tested . The virus was successfully reisolated, so there is little chance that this agent was a laboratory contaminant .

A summary of the patients serological response to the isolate and other Flaviviruses as measured by hemagglutination inhibition (HI), CF and NT is presented in Table 2 . On day 8 of illness, the patient had low CF antibody levels to Bussuquara (BSQ), Ilheus (ILH) and Rocio (ROC) viruses, but nothing to the other agents tested . Subsequent serum samples showed a broad serological response by HI and CF to all Flaviviruses tested, including the isolate . This response is typical of a secondary response to previous Flavivirus exposure . By NT the patient developed a higher titered response to yellow fever (YF) virus than to the isolate , and her sera only partially neutralized the Belém prototype of SLE virus .

The patient reported that she had not left Belém in the month prior to becoming ill . At the time of onset of symptoms, she was working as a maid in a housing development about 1 Km from the Belém airport . A secondary growth forest crossed by a small stream lies about 100 M from the house in which she worked . Epidemiological investigations were begun in late October 1978 in this development in an effort to detect the source and extent of illness in the community. The house in which she had worked and lived was found to be infested with rats , and numerous containers of stagnant water were found in the yard . A total of 28 persons resident in the area, including household contacts, were bled and their sera examined for the presence of HI antibody to YF, BSQ, ILH, ROC, SLE and Be An 327600, a new unregistered Flavivirus from Brazil . Only 5 sera reacted positively . Two sera showed specific reactions only with ILH virus, one specifically inhibited SLE virus, and another only YF virus, while the fifth reacted with most Flaviviruses tested . The only high titered reaction seen was in a serum positive to ILH virus at 1 : 160 ; all other positive reactions were low titered .

Human bait mosquito collections were made from 2000–2400 hrs for 5 nights during late October and early November around the house where the patient worked, and around nearby houses . A total of 656 insects representing 6 species were captured . The majority of these were Culex quinquefasciatus mosquitoes . No virus could be recovered from these arthropods when they were assayed as 47 pools in Vero cell culture . Numerous small wild birds belonging to the families Cuculidae, Thraupidae, Tyrannidae, Trogodytidae and Pipridae frequent the

surrounding area, but no attempt was made to capture any for virological or serological studies .

This is the second recorded isolation of SLE virus from a person in Brazil .

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ANTIBODY SURVEY OF TWO ARENAVIRUSES AMONG LABORATORY PERSONNEL AT THE IEC .

In a previous report (Arthropod-borne Virus Information Exchange nº 35) we described a probable laboratory infection with Be An 293022 (Flexal) virus a new member of the arenavirus group . In view of this finding, it was decided to exam sera of laboratory personnel for the presence of antibody to this agent . A totalof 55 persons working at different levels of exposure to the agent were bled in November 16 - 17, 1978 and their sera tested for the presence of complement fixing (CF) antibodies to Flexal and Amapari viruses, the two arenaviruses found in the Amazon region of Brazil . Below are shown the results among persons at risk, who had or had not handled the agent .

Workers	Pos/tested
Handled virus 2 - 20 times	2/10
No handling of virus	1/45

Next are shown the CF titers found to Flexal and Amapari viruses in these 3 serum samples, including titers found on serum specimens collected from 2 of the positive workers prior to November 78 and on the patient's serum from Flexal virus was isolated .

Antigen mouse br. suc.acet.	Persons by registration number and dates of serum collection							Homologous
	Bel 4435 + Nov.17 1978	Bel 13060 May30 1978	Bel 13060 Nov16 1978	Bel 13058 Dec20 1977	Bel 13058 Nov16 1978	Bel 14066 Nov16 1978	Bel 14066 Nov22 1978	
Amapari	0	8	8	0	4	8	8	≥ 32
Flexal	32	8	8	0	4	8	8	≥ 32
Piry	0	0	0	0	0	0	0	>16

+ Serum drawn about 9 months after isolation of Flexal virus from this person .

The presence of low CF antibody titers to Flexal virus in the sera of the 3 laboratory workers is difficult to interpret . None of them reported disease similar to that exhibited by the single case (Bel 4435) of Flexal virus infection during the period of exposure to this agent . It would be of interest then to check if their sera neutralize Flexal virus ; however, to date we have been unable to develop a neutralization system for this agent .

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OUTBREAKS OF YELLOW FEVER AND OROPOUCHE VIRUS DISEASE IN TOMÉ AÇU, PARÁ, 1978 .

An outbreak of yellow fever (YF) occurred in the rural area of Tomé - Açu county, Pará State, Brazil in 1978 . The county is located some 120 Km southeast of Belém . According to the mayor it has 45.000 inhabitants . SUCAM' s data indicate populations of 4340 persons in the county seat and 2280 in the village of " Quatro Bocas ", the two main urban centers . From June until December 10 cases of the disease, 6 of which were fatal, have been confirmed by laboratory tests . Seven additional fatal cases were diagnosed on clinical and

epidemiological grounds . In the 6 fatal cases confirmed by laboratory examination the diagnosis was made histopathologically (4 cases), by virus isolation (1 case) or serological conversion (1 case) . It is noteworthy that the latter patient (IRI 111) died on the 24 th day of illness . He was a 23 y.o. male native of Irituia an county adjoining to Tomé - Açu, where he was working at the time he developed symptoms . His death was compatible with renal failure, since levels of urea and creatinine in the blood were extremely high . Albuminuria was also present . Surprisingly, no histopathological lesions could be detected in a liver specimen taken after death . A rise in HI antibody titers to YF virus and other flaviviruses known from Brazil was observed during the illness (Table 3) . The 4 cases surviving were confirmed by virus isolation (3 cases) and by serological conversion (1 case) .

The monthly distribution of the 17 cases by time of onset was as follows : June (9), July (5), August (1) , October (1) and November (1) . All the 17 patients were males and ages varied from 7 to 48 years old . All of them had frequented forest .

Although YF virus could not be recovered from 45 Haemagogus sp captured in the area and inoculated into infant mice as 2 pools, this was a typical outbreak of jungle YF . In passing, it should be noted that Aedes aegypti is not present in the Amazon region of Brazil .

During the search for cases of YF, two serological conversions to Oropouche (ORO) virus were detected by IH test, in patients from Tomé-Açu county . One case (TA 663 from Canindé) was first seen on July 5 and the other one (TA 752 from Quatro Bocas) on July 18 . There was also a patient (TA 754) with a titer of 1 : 320 to ORO virus in a serum sample collected on July 5 .

These findings encouraged us to investigate the possible occurrence of an outbreak due to this agent . Active search for cases was made during August-September in "Quatro Bocas " and nearby localities . As result of this ORO virus was isolated from 23 of 68 febrile cases examined . In addition, 4 cases (including the two above) were recorded serologically . Patient's age varied from 2 to 50 . Twenty were males and 7 were females .

Clinical manifestations were similar to those observed in the past, namely , fever, chills, headache, myalgias, arthralgias, dizziness . The cases in general were mild, however , a few patients became severely ill and were hospitalized . Viremia measurements revealed that average titers of virus was 4-5 log /ml (Vero, hamster, mouse) during the first 3 days of illness , then fell to low levels (1-2 log) on the 4 th day , and became negative on the 5 th day .

Interestingly, seven of our workers in Tomé-Açu became sick and yielded ORO virus from blood . The number of IEC workers that visited Tomé-Açu during the outbreak, according their duties, was as follows :

Team	Pos virus / total exposed	% Pos
Entomology	4/8	50 %
Epidemiology	1/7	15 %
Ecology	1/2	50 %
Drivers	1/4	25 %
Total	7/21	33 %

The density of Culicoides paraensis was " high " in Quatro Bocas and "extremely high " in nearby cacau & black pepper plantations .

HI antibodies to ORO virus was detected in 6 (1,6 %) of the 370 wild birds (mainly Fringillidae Thraupidae Columbidae and Tyrannidae) . HI tests were negative for ORO virus with plasma or sera obtained from 139 domestic fowl , 17 bats and 1 canivore .

Studies are on progress to determine the post-epidemic immunity rate to ORO virus in differents localities of Tomé-Açu .

Although the YF and ORO virus outbreak were simultaneous, it was clear that they were occurring at different places in the county . Thus, most of the YF infections was acquired in the forests or near them, whereas most ORO virus infections seem to have occurred in urban and rural environments .

Francisco P. Pinheiro, Amelia P.A. Travassos da Rosa, Jorge F. Travassos da Rosa , Alexandre C. Linhares, Yvone B. Gabbay . Evandro Chagas Institute, FSESP , Brazilian Ministry of Health .

Table 1

Identification by complement fixation (CF) and neutralization tests (NT) of virus BeH 355964 isolated from a jaundiced patient serum in Belém, Pará, Brazil, October 1978 .

Antigen	C F M.H.A.F. +							N T M.H.A.F.		
	BeH 355964	SLE	YF	BSQ	ILH	ROC	Patient's serum day 29	SLE	YF	Patient's serum day 29
Be H 355964	128[†]	128	0	0	8	16	128	1,5§	0,2	2,5
St. Louis encephalitis (SLE)	128	128					128	2,6	≤0,5	0,8
Yellow fever (YF)			≥64				128	1,5	5,2	3,0
Bussuquara (BSQ)				≥64			128			
Ilhéus (ILH)					≥64		128			
Rocio (ROC)						≥64	64			
Be An 327600 "							64			

\+ M.H.A.F. = mouse hyperimmune ascitic fluid

† Reciprocal of serum dilution

• 0 = < 1 : 4

§ $\log_{10}$ of neutralization index

" New Flavivirus for Brazil, isolated in the Amazon region

Table 2

Serological response by hemagglutination-inhibition (HI), neutralization (NT) and complement fixation (CF) tests of patient to virus Be H 355964 and various other Flaviviruses by day of illness .

Antigen	Patient' s sera (days of illness)														
	H I					N T					C F				
	8	18	29	49	124	8	18	29	49	124	8	18	29	49	124
BeH 355964	0+	320++	320	160	80	0	1,7+++	2,5	2,7	1,2	0	128	128	64	8
SLE "	0	320	320	160	80	0	1,4	0,8	0,8	1,8	0	128	128	64	16
Yellow fever	0	320	320	160	80	1,2	2,7	3,0	2,8	2,5	0	128	128	64	8
Bussuquara	0	160	160	80	20	0,1				0,2	16	.128	128	64	8
Ilhéus	0	320	320	160	80						8	128	128	64	8
Rocio	0	160	160	80	40	0				0,3	16	128	64	32	0
BeAn 327600■	0	160	160	80	40						0	64	64	32	16

" Prototype strain of SLE virus isolated from mosquitoes collected in Northern Brazil

■ A new Flavivirus to Brazil

\+ 0 = <1 : 8 (CF) <1 : 10 (HI)

++ Reciprocal of serum dilution

+++ Log_{10} of neutralization index

Table 3

HI antibody response of patient IRI 111 to flaviviruses . Tomé-Açu, 1978 .

Patient's serum (day of illness)	V I R U S					
	YF	BSQ	ILH	SLE	ROC	An 327600 +
9	160	80	80	80	80	≥20
12	640	640	1280	640	640	640
16	640	640	1280	640	640	640
19	640	640	1280	640	640	640

+ New flavivirus to Brazil .

REPORT FROM THE ARBOVIRUS LABORATORY

INSTITUT PASTEUR AND O.R.S.T.O.M.

B.P. 304

97300 - CAYENNE, GUYANE FRANCAISE

DETECTION OF THE DENGUE VIRUS WITH THE FLUORESCENT FOCI METHOD LLCMK2.

This technique of isolation was reported in the March 1978 Information Exchange (Number 34, p.197) as used during the 1977 dengue outbreak in Cayenne. Eighteen strains were isolated from human sera and four from mosquitoes. All strains were dengue type 2.

From March to September 1978, clinical cases of dengue occurred in Cayenne and 23 strains were isolated from human samples. The virus was identified as dengue type 1. This was the first time dengue 1 has been identified in French Guiana.

A slight modification of the technique previously described was used : acute human serum was inoculated directly onto LLCMK2 cells grown in Lab Tek TC 8 chambers slides and into Aedes aegypti. On the third day the slides were stained by indirect fluorescence method, using a broad reacting anti dengue 2 mouse ascitic fluid and a fluorescein labelled anti mouse gamma globulin serum.

If specific fluorescent foci were observed, the nex day inoculated mosquitoes were ground, the suspension diluted 1 : 100 and neutralized by dengue 1, 2, 3 mouse ascitic fluid at the optimal dilution.

The type of the virus was identified by fluorescent focus reduction . Using this method, we have been able to identify dengue 1 virus on the seventh day following the collection of the serum.

J.P.DIGOUTTE, G. GIRAULT, M. LHUILLIER, Y.ROBIN

REPORT FROM GORGAS MEMORIAL LABORATORY

PANAMA CITY, PANAMA

I. St. Louis Encephalitis Virus in Mansonia dyari

Although widespread distribution of St. Louis encephalitis (SLE) virus in Panama has been shown from sporadic virus isolations in several species of sylvan mosquitoes, man and birds and from antibodies found in man and vertebrates, nine strains of SLE were isolated for the first time in 1977 from pools of Mansonia dyari, formally called indubitans (Information Exchange #33). This burst of viral activity in the Bayano field station included infection of sentinel monkeys, hamsters and chickens and was associated with an overgrowth of Pistia stratiotes (water lettuce) in the area. Since M. dyari had never been implicated in transmission of SLE, it was important to determine vector competence of this sylvan species.

We tested wild-caught populations for their susceptibility to SLE infection by artificial and natural means and their ability to transmit virus to baby chicks. Mosquitoes were infected by intrathoracic inoculation of viral suspensions containing 4.5 to 8.0 DEX $TCID_{50}$/ml of SLE strain 4336 (original isolated from M. dyari pool from Bayano). Mosquitoes infected by ingestion fed either on pledgets soaked in blood-sucrose-virus suspensions of the same concentrations or on viremic cormorants and herons previously infected with 0.2 ml of 2000-5000 PFU/ml of virus. Virus antigen in head squashes was detected after eight days or more by indirect fluorescent antibody (IFA) tests and virus assay of mosquito bodies were made in Vero cell fluid and overlay cultures.

Infection rates are shown in Table 1. Less than 50% of the mosquitoes feeding on virus levels of 5.5 to 7.0 DEX/ml became infected. Threshold values were apparently way below that of the lowest concentration of virus in the pledgets. With viremic birds as the virus source, mosquitoes became infected after feeding on herons and cormorants circulating 1.7 to 4.5 DEX/ml of virus. Infection rates by inoculation were high as expected. Transmission of SLE to chicks occurred mainly with mosquitoes originally infected by inoculation; a single mosquito infected by ingestion of 4.5 to 5.0 DEX/ml of virus transmitted SLE to a chick. Virus was detected in chick plasmas on days 2 and 3; month-old chicken sera have yet to be tested for antibody.

This is the first time the vector competence of M. dyari for SLE has been studied. We obtained data regarding infection and transmission rates which, when compared with rates of known SLE vectors in the United States, indicate that M. dyari is not a very efficient vector. However, we cannot be certain that the stresses of abnormally high populations of a less-than-efficient vector might, nonetheless, influence viral activity.

(H. G. Wallace and P. Galindo)

II. St. Louis Encephalitis Virus in Herons

Vertebrates including birds that have demonstrated antibodies to SLE are likely suspects as reservoir and/or amplifying hosts in the transmission of SLE in a tropical forest habitat. Previous antibody surveys have shown that herons were easily infected with SLE and were very possible candidates. Viremic patterns in the striated heron, Butorides striatus, were studied to determine the importance of these birds in the transmission of SLE in Panama.

Eight nestling and fledgling herons were bled and tested seronegative before use. About 0.2 ml of 2000-5000 PFU/ml of SLE virus strain 4336 was inoculated subcutaneously into skin folds near or around the beaks and eyes of each bird. Heparinized blood samples were taken every day post-inoculation until day 7; all samples were stored at Revco temperatures until tested simultaneously in Vero cell fluid and overlay cultures. Blood serum samples drawn after 3 to 4 weeks were tested for HI antibodies.

Table 2 summarizes viremia data for seven herons; one died of unknown causes on day 4. All other herons showed no signs of illness and developed HI antibodies. Viremias were somewhat low in titers but lasted three to five or more days. Nestling viremias were at least one day longer. It is significant that viremic patterns of such duration and titers were adequate to infect Mansonia dyari mosquitoes feeding on the herons on days 2 and 4.

(H. G. Wallace and P. Galindo)

III. Infection of Toxorhynchites Mosquitoes with Some Arboviruses

Two laboratory colonies of Toxorhynchites species, T. amboinensis and theobaldi, were infected with various arboviruses. T. theobaldi is the only colonized species from Central and South America.

Infection of T. amboinensis with SLE, Gamboa and VEE viruses showed that virus replication in male mosquitoes was similar to that in females in almost all instances when samples taken routinely at days 7, 10, 14 and 21 were tested. Peak titers for all viruses in mosquitoes tested were reached mainly on day 7. Plateaus occurred from that point on with titers dropping only 1.0 to 2.0 DEX/ml or less. VEE titers and virus yields in T. theobaldi were comparable. Because virus yields ranged from approximately 10 to 10,000 fold increase, producing high-titered virus stocks in this mosquito tissue "milieu" would be of great practical value.

Both species were inoculated with two dengue virus strains: DEN-1 Hawaiian and DEN-1 Jamaica. Virus antigen in head squashes was detected as early as the 6th day by IFA tests using DEN-1 hyperimmune ascites fluid and fluorescein conjugated anti-mouse rabbit serum.

Dengue viruses were recently recovered for the first time in this laboratory from T. amboinensis and theobaldi inoculated with acute human sera.

Twenty sera of 38 tested were found IFA positive. At least seven sera negative by IFA were determined positive after passage of sonified mosquito bodies into cell culture systems.

(H. G. Wallace, J. Petersen and P. Galindo)

Table 1: Infection of Mansonia dyari With SLE Virus

Virus Titer in DEX $TCID_{50}$/ml	Percent of Infected Mosquitoes* by Inoculation	Pledget Feeding	Viremic Birds
Herons			
1.7			31 (5/16)
2.3			11 (2/18)
Cormorants**			
2.3			17 (2/12)
4.5			25 (2/8)
Suspensions			
4.5 to 5.0	88 (28/32)	26 (11/42)	
5.5 to 6.0	94 (16/17)	47 (7/15)	
6.5 to 7.0	93 (39/42)	44 (8/18)	
7.5 to 8.0	100 (4/4)	71 (5/7)	

* No. of mosquitoes infected/No. of mosquitoes engorged
** Virus titer in PFU/ml

Table 2: SLE Viremia Patterns in Striated Herons (Butorides striatus)

Bird	Duration of Viremia in Days			Peak Titers of Viremia		
	Min.	Max.	Mean	Min.	Max.	Mean
Nestlings (PFU/ml)	4	≥ 5	≥ 4.5	1.3	3.3	2.3
Fledglings (DEX $TCID_{50}$/ml)	3	4	3.5	1.5	4.5	3.0

IV. A New Equine Cell Line

We have established a new cell line designated GML-232. It originated from a 71-day old fetal equine liver explant culture. The explant was initially infected with VEE strain P-676 which disappeared within one week of initiation of the cell line. GML-232 is currently at passage 43. It remains free of VEE virus, bacteria, fungus and mycoplasma. GML-232 consists of epithelioid cells; has a fast growth rate requiring weekly subculture at a 1:10 split ratio; is non-tumorigenic; has a modal chromosome number of 55. These cells appear highly susceptible to a wide variety of viruses (Table 3).

Table 3: Virus Susceptibility of VERO and GML-232 Cells

Virus	$TCID_{50}$/ml	
	Vero	GML-232
Yellow fever - Wild strain	$10^{7.3}$	$10^{7.5}$
Yellow fever - 17D strain	$10^{6.5}$	$10^{6.3}$
VSV - Indiana	$10^{6.7}$	$10^{7.8}$
VEE - 3880	$10^{7.3}$	$10^{7.7}$
St. Louis encephalitis	$10^{7.7}$	$10^{7.7}$
Polio type 3	$10^{7.5}$	$10^{7.0}$
Coxsackie B-1	$10^{7.5}$	$10^{7.7}$
Measles	$10^{5.7}$	$10^{6.7}$
Herpes simplex type 1	$10^{6.7}$	$10^{7.0}$
Herpes simplex type 2	$10^{4.7}$	$10^{3.0}$

(G. Oro)

V. Human VEE Outbreak

Between August and October 1978 an outbreak of febrile illness occurred in Chilibre, Panama. Chilibre is a rural community located close to the Chagres River approximately 20 Km. from the capital. This area was first documented as an enzootic VEE focus (Subtype I-D) in 1962.

We received specimens from 36 patients (29 were less than 15 years old). Most patients complained of chills, headache, myalgia and gastrointestinal disturbances (nausea, vomiting, diarrhea and/or abdominal pain). Several also presented with upper respiratory symptoms (pharyngitis, amygdalitis, cough).

VEE virus was isolated from 3 patients. All three had positive throat swabs and one was also viremic. Isolation attempts on another 23 patients, seen within the first five days of illness, were negative for VEE virus (18 throat swabs, 23 sera).

Plaque neutralization tests using Panama VEE strain 3880 (Subtype I-D) revealed that 3 patients seroconverted (one a patient with an isolate), while 13 remained free of anti-VEE antibody. One patient (lacking an acute blood) showed VEE antibody in his convalescent serum. Two children, six and eight years old, had VEE antibody during the acute phase (days 1 and 3). Another 17 patients (including 2 with VEE isolates) have not yet been tested serologically. Thus 5 of 21 (24%) fully worked-up cases clearly represented VEE infection; three individuals had probably been infected previously with VEE; another 15 showed no evidence of VEE infection. An additional 15 persons remain to be tested.

(P. Peralta, E. Quiroz, R. Saenz, G. Justines)

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

The 1978 Dengue Epidemic in Puerto Rico: Laboratory Observations

The epidemic curve for the 1977 and 1978 epidemics is shown in Figure 1. Serum pairs received through the dengue surveillance program were tested for dengue antibodies. Sera submitted for nondengue diagnostic studies were also tested for dengue. In 1978, there were 3,361 serum pairs from suspected dengue cases, and dengue was confirmed in 77% (2,602). Samples were received from 77/78 municipalities and confirmed in 76/78. The peak of the dengue epidemic occurred in June, when 80% of suspected cases were serologically confirmed as dengue. These serological studies have provided data on the extent of the dengue problem in Puerto Rico and information for mosquito control measures. The possibility of the initiation of a dengue epidemic in the continental United States by returning travelers cannot be overlooked.

Serum samples from suspected dengue cases in the continental U.S. were tested and dengue infections confirmed from 18 states. These infected persons had all traveled or resided outside the continental U.S. during the incubation period, in areas with current dengue infection, including Puerto Rico. No secondary cases were recognized in the U.S.

In FY 1977, over 28,000 mosquitoes were inoculated with 334 sera for fluorescent antibody tests. In FY 1978, nearly 20,000 mosquitoes were inoculated with 855 acute phase human sera. Thus, there was a 150% increase in the number of sera processed with a 29% decrease in number of mosquito inoculations. The use of Toxorhynchites amboinensis mosquitoes in place of A. aegypti has greatly improved the mosquito inoculation technique for virus isolation. Identification is more rapid with the new system. Fewer mosquitoes are inoculated (10 instead of 40), survival is higher, and fewer complement fixation (CF) identification tests have to be repeated. In FY 1978, 417 of the 855 sera inoculated were submitted for CF testing, either as FA positives or as negative controls.

The CF identification of dengue viruses, with infected mosquitoes as antigen, has proved to be a rapid, accurate identification procedure. In every case where identification has also been carried out by plaque reduction neutralization test, the CF test result has been confirmed. This has provided information on the distribution of these viruses and the type active at a given time. During 1978, types 2 and 3 apparently disappeared from Puerto Rico, and practically all isolates from Puerto Rico and elsewhere have been type 1.

The mechanics of producing large numbers of Toxorhynchites continues to be the major limiting factor in the mosquito inoculation system. Large numbers of A. aegypti must be reared to feed the Toxorhynchites

larvae, which are predatory (about 350 A. aegypti larvae needed to raise one Toxorhynchites adult). To reach an average production of 100 Toxorhynchites per day, we need to produce over 250,000 A. aegypti per week.

Computerization of the laboratory and clinical data from 1978 is not complete, nor is testing of the population surveys, so that analyses of clinical and epidemiological parameters of the 1978 epidemic are not yet possible. A summary of data on the two epidemics of 1977 and 1978 is given in the following table:

DATA ON 1977 and 1978 EPIDEMICS

	Dengue Epidemic	1977	1978
A.	No. patients reported	12,733	12,965
	No. patients tested	3,394	3,361
	No. laboratory confirmed	1,358	2,602
	No. viruses isolated	98	346
	Dengue virus types involved	1,2,3	1
	Estimated clinical attack rate	7%	14%
	Estimated no. clinical cases	224,000*	450,000*
	Peak age	20-29	20-29
	Sex ratio, male:female	0.67	0.82
	Rate of hemorrhagic manifestations	10%	NA**
	No. patients seen with serious complications	6	3
	No. deaths	0	1
B.	No. ground ULV spray machines	9	17
	Estimated cost of ground control	$439,000	$710,000
	Cost of air spraying	$300,000	$180,000
	Estimated economic loss	$6,000,000+	NA**

*1977 estimated population of Puerto Rico was 3.3 million.

**Not yet available

Aedes aegypti Surveillance in Relation to Dengue Virus Activity in Puerto Rico, 1978

A. *Aedes aegypti* population changes during 1978

As in previous years, the availability of long-term, base-line data on *A. aegypti* abundance has permitted us to judge the effectiveness of various control operations. Most of the 1978 data are not yet analyzed. Only a few of the most important data are discussed here.

Long-term larval surveillance. Larval indices were measured in four cities--Guayama, Ponce, Mayaguez, and Arecibo. Average larval indices were about the same as in 1977, with the possible exception of Ponce.

Adult *A. aegypti* abundance. Ten modified New Jersey traps were operated on a regular basis during 1978 (Figure 2). Traps were run 6 hours per day (0800 to 1400 hours) from January through July, after which they were operated on a 24-hour/day basis. The use of a glycerine and alcohol solution in the collecting jars allowed weekly instead of daily pickup of samples.

The modified New Jersey traps and the "CDC Sweeper" were compared in Arecibo. The two sets of collections were not significantly different. Average daily collections in five cities varied from 2.0 to 8.1 female *A. aegypti* per trap day (Table 1).

B. Predictive studies with *A. aegypti*

Most of the 1978 data have not been analyzed. However, the observed larval indices for the Ponce study area were 44% lower than those predicted by our rainfall model. This suggests that the various types of control being used throughout the island are having a measurable effect on *A. aegypti*.

C. Evaluation of *A. aegypti* source-reduction activities

Elimination of *A. aegypti* breeding sites is probably the most effective control method available. However, evaluation of source-reduction activities is a difficult and complex task. In addition to knowing how much material has been removed, we want to know whether the actual number of breeding sites has been reduced. Pre- and post-cleanup larval inspections are made to estimate percent reduction in positive breeding sites. At the same time, some external control is needed to show natural changes in breeding, especially changes related to rainfall. In this case, *A. aegypti* breeding in cemeteries has been used as the external control.

Pre- and post-cleanup inspections. Tables 2 and 3 show summary data for changes in total potential breeding sites and for those actually containing larvae. The percent reduction in containers with larvae was considerably greater than the overall percent reduction in containers. This difference could be due to several factors. First, changes in numbers of rain-filled containers between pre- and post-cleanup inspections will affect the number of positives. Also, there may be a "sensitization" effect from the public education program such that residents regularly empty water from potential breeding sites.

Cemetery surveillance. The average percent of cemetery flower urns found infested with A. aegypti (100 urns examined each week) in the eight health regions varied from 4 to 31. Due to apparent concentration of suspected dengue cases around some of the cemeteries, control with malathion and Abate^{R} was instituted at the end of September (weeks 39-40). Ovitraps were placed in the cemeteries and in nearby houses. In general, the data from ovitrap collections do not show a close correlation between breeding within cemeteries and in nearby houses.

(J. P. Woodall, R. H. López-Correa, C. G. Moore, G. E. Sather, G. Kuno, F. K. Banegura) San Juan Laboratories, CDC, GPO Box 4532, San Juan, Puerto Rico 00936

TABLE 1
SUMMARY DATA FOR TEN MODIFIED
NEW JERSEY TRAPS IN FIVE PUERTO RICAN CITIES, 1978

Parameter	Arecibo	Mayaguez	Ponce	San Juan	Bayamón	Total
No. of traps	3	2	1	2	2	10
No. of trap days	566	351	113	374	419	1,861
Percent utilization	52	48	31	51	57	49
Females per trap day	2.0	2.5	3.7	8.1	2.5	3.6

TABLE 2
PERCENT OF POTENTIAL BREEDING CONTAINERS
PRE- AND POST-CLEANUP INSPECTIONS

Total cleanup campaigns evaluated----------38

	Pre	Post	Percent change
Total premises inspected	3,916	3,848	---
Total containers	36,420	26,659	-27
Total containers with water	7,456	5,711	-24
Total containers with larvae	851	362	-57

TABLE 3
NUMBERS AND PERCENT REDUCTION IN DIFFERENT
TYPES OF CONTAINERS, PRE- AND POST-CLEANUP

Type of containers	Total Containers Pre	Post	Percent change
Tires	1,855	774	-59
Small miscellaneous	6,810	4,487	-35
Tin cans	10,815	6,979	-36
Large miscellaneous	1,953	1,562	-21
Bottles	7,217	5,766	-21
55-gallon drums	1,165	956	-18
Buckets	3,277	2,888	-12
Animal drinking containers	3,328	3,247	- 3
Total containers	36,420	26,659	-27
Containers with Larvae			
Tires	267	62	-77
Small miscellaneous	112	104	- 7
Tin cans	119	40	-66
Large miscellaneous	23	22	- 4
Bottles	28	6	-79
55-gallon drums	96	40	-58
Buckets	96	44	-66
Animal drinking containers	110	44	-60
Total with larvae	851	362	-57

FIGURE 1

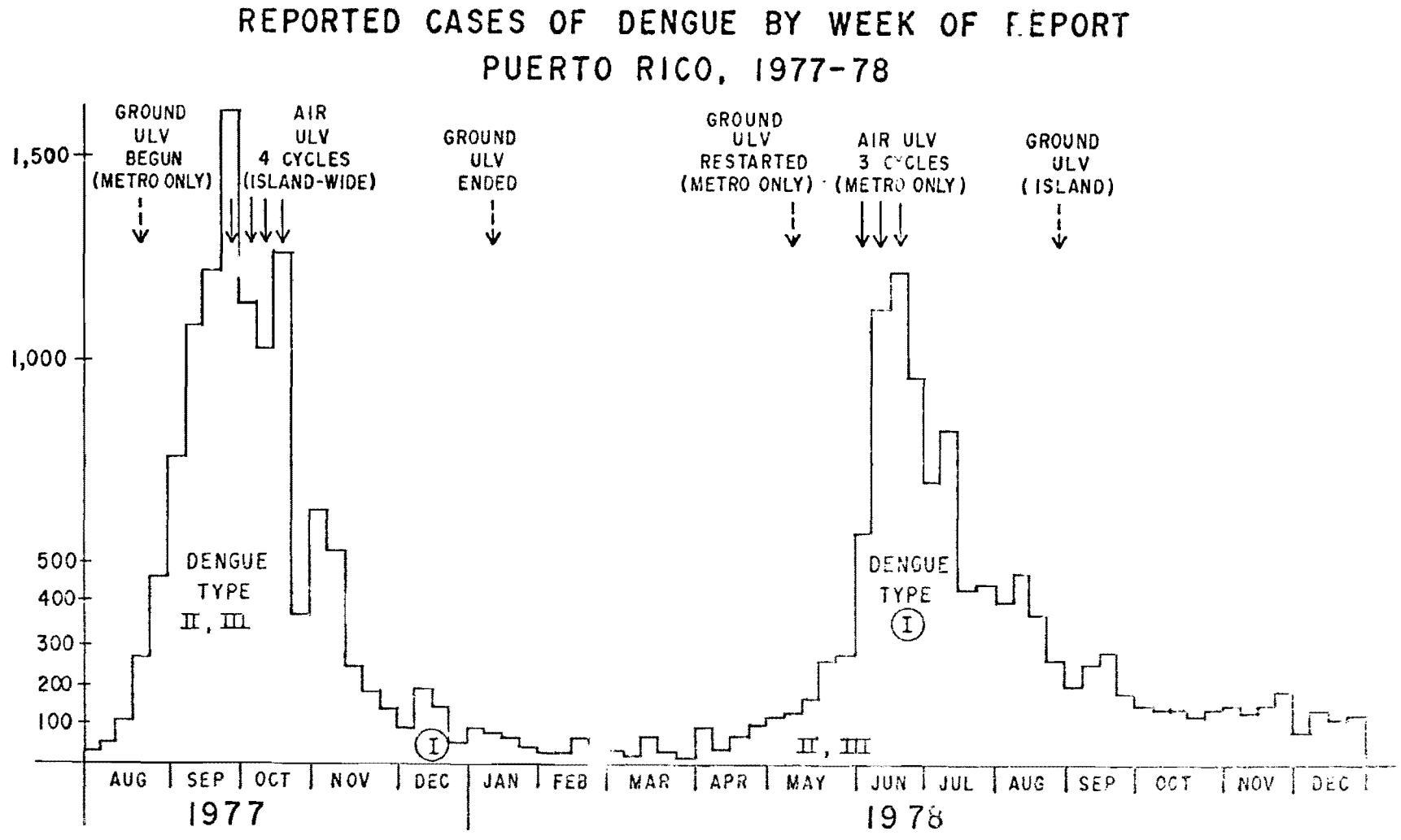
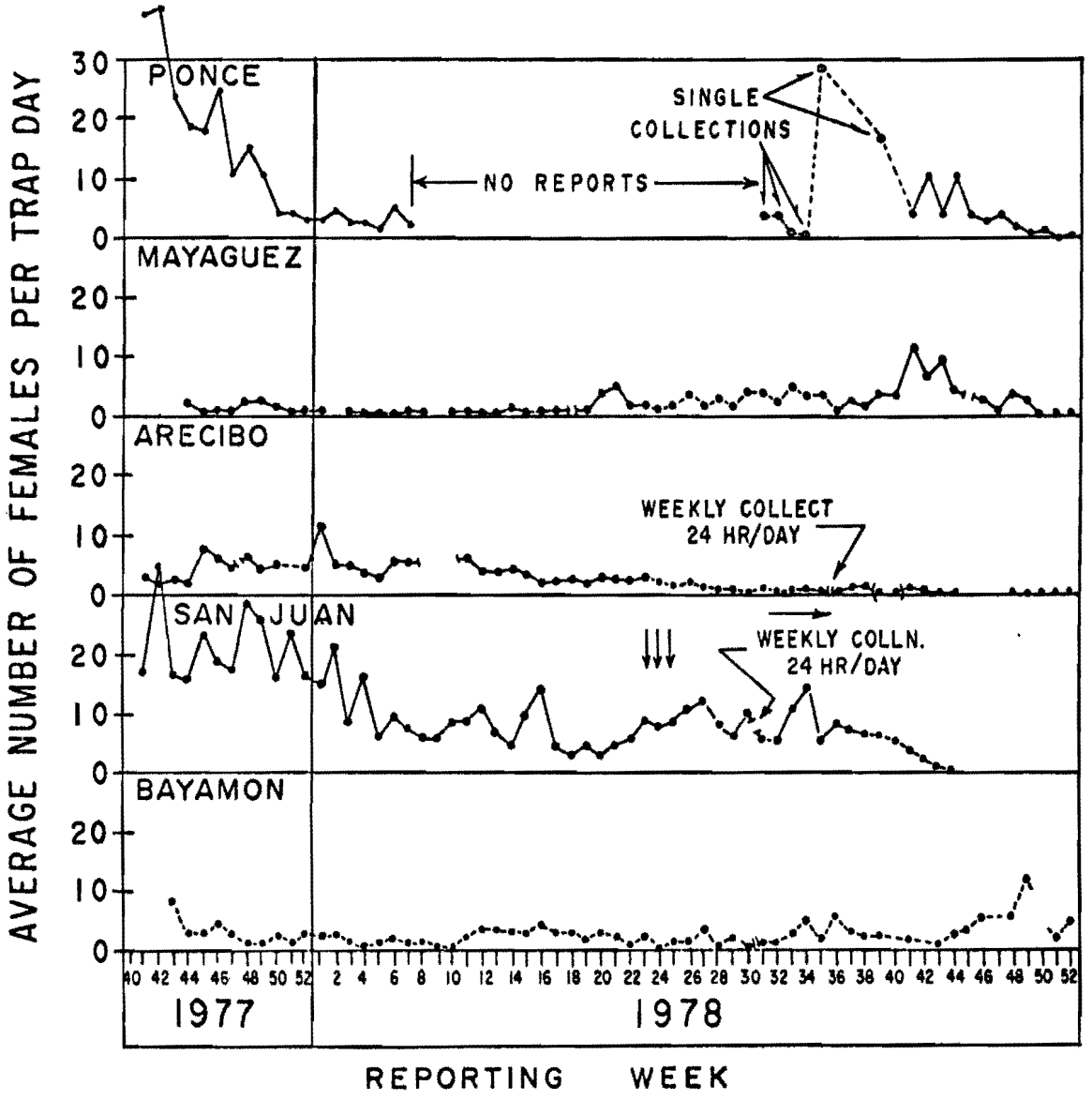

REPORTED CASES OF DENGUE BY WEEK OF REPORT
PUERTO RICO, 1977-78
GROUND ULV BEGUN (METRO ONLY)
AIR ULV 4 CYCLES (ISLAND-WIDE)
GROUND ULV ENDED
GROUND ULV RESTARTED (METRO ONLY)
AIR ULV 3 CYCLES (METRO ONLY)
GROUND ULV (ISLAND)
1,500
1,000
500
400
300
200
100
DENGUE TYPE II, III
DENGUE TYPE I
II, III
I
AUG SEP OCT NOV DEC JAN FEB
1977
MAR APR MAY JUN JUL AUG SEP OCT NOV DEC
1978

FIGURE 2

AVERAGE DAILY CATCH OF FEMALE AEDES AEGYPTI IN MODIFIED NEW JERSEY TRAPS, 1977-1978.



REPORT FROM THE TEXAS DEPARTMENT OF HEALTH
AUSTIN, TEXAS 78756

During the period July 7, 1978-December 31, 1978 a total of 29,832 mosquitoes were tested for arboviruses. Listed below are the positives:

Locality	Collection Date	Species	# Isolations	Virus
Hidalgo Co.	6-5-78	C. quinquefasciatus A. aegypti	1	Hart Park
Dallas Co.	6-13-78	C. quinquefasciatus C. restuans	1	Hart Park
Calhoun Co.	6-13-78	C. quinquefasciatus C. quinquefasciatus P. cyanescens	1	Hart Park
Wichita Falls	6-14-78	C. quinquefasciatus A. vexans	1	Hart Park
Tarrant Co.	6-20-78	C. quinquefasciatus C. restuans C. salinarius C. territans	1	Hart Park
Cameron Co.	6-20-78	C. quinquefasciatus A. quadrimaculatus C. quinquefasciatus C. quinquefasciatus	1	Hart Park
Hidalgo Co.	6-20-78	A. aegypti C. quinquefasciatus A. aegypti	1	Hart Park
Dallas	7-13-78	C. quinquefasciatus C. quinquefasciatus A. pseudopunctipennis	1	Hart Park
Port Arthur	7-18-78	A. quadrimaculatus A. crucians	1	Tensaw
Hidalgo Co.	7-17-78	C. quinquefasciatus A. aegypti C. quinquefasciatus C. quinquefasciatus	1	Hart Park
Public Health Region I	7-18-78	C. tarsalis C. tarsalis A. vexans	1	Hart Park
Dallas City	7-21-78	C. quinquefasciatus C. restuans Culex species	1	Hart Park

Public Health Region I	7-31-78	C. tarsalis A. vexans	1	Hart Park
El Paso City	8-15-78	C. tarsalis A. vexans	1	WEE
Cameron Co.	8-22-78	A. sollicitans A. taeniorrhyncus A. bimaculatus	1	CEV
Brazoria Co.	8-29-78	C. quinquefasciatus A. quadrimaculatus A. quadrimaculatus	1	Tensaw
Calhoun Co.	8-30-78	A. sollicitans A. taeniorrhyncus P. columbiae P. discolor	1	Tensaw
Dallas Co.	10-10-78	C. quinquefasciatus C. tarsalis C. restuans	2	Hart Park Turlock

The following regions submitted sera for arbovirus surveillance. HI's were performed on all these: Canyon, Lubbock, Marfa, Arlington, Dallas City, Dallas County, Tyler, Willacy and Cameron County, Uvalde, and San Antonio. Positives are listed below:

Locality	Collection Date	Species	# Positives	Antibodies detected
Lubbock	10-12-78	Chickens	5	4 SLE 1:20 1 VEE 1:20
	9-12-78	Canine	3	VEE 1:20
	9-19-78	Canine	1	SLE 1:20
Marfa	?	Canine	1	WEE 1:80
Tyler	10-4-78	Horse	3 1	WEE 1:40 VEE 1:20
Willacy Co.	9-29-78	Canine, Feline	1	WEE 1:20
Cameron Co.	9-19-78	Equine	3	2 SLE 1:20 1 WEE 1:20
	10-19-78	Chickens	2	WEE 1:20
	10-10-78	Equine	2	VEE 1:20
	11-21-78	Equine	8	VEE 1:20 WEE 1:20

A total of 3415 sera were submitted and tested. Animals included sentinel chickens, wild birds, canines, felines, equines.

(Charles E. Sweet and staff of the Medical Virology Branch)

REPORT - JULY 1-DECEMBER 31, 1978
OFFICES OF LABORATORY SERVICES AND ENTOMOLOGY
DEPARTMENT OF HEALTH AND REHABILITATIVE SERVICES, JACKSONVILLE, FLORIDA

Extensive surveillance in Florida during 1978 failed to elicit any evidence of SLE activity in the State. However, increased EEE activity was noted between June and October. Surveillance results are categorized below.

Human - Sera from 911 patients with CNS symptoms were subjected to HI antibody tests against EEE, SLE, VEE, Dengue 2, and CAL antigens. One 54-year-old male had a fatal case of EEE. Seven of the 911 patients were confirmed as Dengue 2, six were apparently infected in Colombia, South America and one in Puerto Rico.

There were 25 patients with constant SLE and/or Dengue titers probably due to the 1977 SLE outbreak in Florida, previous flavivirus infections, and/or immunizations. A missionary from Honduras had a constant EEE titer.

Avians and Mammals - A total of 5,550 sera were tested for SLE and EEE HI antibodies. A breakdown of the results is shown below. Conversions to EEE were documented but no evidence of SLE activity was found.

Serum Source	Number Tested	Number Positive SLE	Number Positive EEE
Sentinel Chickens	4,127	0(1)	83(2)
Backyard Chickens	225	1(3)	0
Wildcaught Avians	538	15	7
Raccoons	218	45	13
Opossums	279	46	17
Exotics	101	2	1
Equines	8	5	7
Fox	3	1	0
Feline	9	1	0
Rodent	36	2	1
Canine	1	1	0
Armadillo	5	2	0

(1) There were 27 reactors which failed to confirm on a repeat bleeding.
(2) Represents 46 conversions between June and September.
(3) Age not known.

Isolation Attempts - All 1,585 pools of Culex nigripalpus mosquitoes tested were negative. Of 253 mammalian tissues tested, CNS tissue from one of three equines yielded an EEE virus.

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

REPORT FROM THE VIRAL DISEASES DIVISION (VDD)
BUREAU OF EPIDEMIOLOGY
CENTER FOR DISEASE CONTROL, ATLANTA, GEORGIA

SURVEILLANCE FOR HUMAN ARBOVIRUS INFECTION, UNITED STATES, 1978

During 1978 arboviral encephalitis activity in the United States was relatively scattered with no major focal outbreaks. As a result of a continuing epidemic dengue in Puerto Rico and a sharp outbreak of dengue in Tahiti, a number of persons returning to the continental United States developed confirmed cases of dengue. The attached table shows the number of laboratory confirmed encephalitis infections and dengue importations by state. Comments on the highlights for specific etiologies are listed below.

California Encephalitis

A total of 144 cases of California group encephalitis infections were reported from 12 states, more cases than for any other year except 1975 (163). An investigation in response to a possible outbreak of CE in southeast Minnesota and southwest Wisconsin revealed evidence of continuing high attack rates in rural children from these areas and resulted in approved regional surveillance and development of coordinated plans for control. The total of 87 cases from the tri-state area (Iowa, Minnesota, and Wisconsin) is the largest annual total on record for the area, accounting for 60% of the U.S. total. Factors in the increased number of cases may include an actual increase in CE activity in the area, results of publicity following a child's death from CE, and increased case ascertainment. An unusually large number of cases (23 including 1 death) was reported from New York, the largest annual total recorded for that state.

St. Louis Encephalitis

After 3 years of major outbreaks, a total of 23 mostly widely spread St. Louis Encephalitis cases were reported in 1978, The only cluster of SLE cases was detected in late fall in a Birmingham, Alabama neighborhood and control activities uncovered and quickly eliminated the apparent source of the infected mosquitoes.

Eastern Equine Encephalitis

The total of 5 human cases of Eastern Equine Encephalitis were limited to scattered locations in Florida. Two of the cases were fatal.

Western Equine Encephalitis

Only 4 cases of Western Equine Encephalitis were reported, all in residents of Minnesota.

Dengue

A total of 89 confirmed dengue cases were imported into 19 of the United States in 1978. The major sources of infection were Puerto Rico, other Caribbean and Central American countries, and Tahiti. The source of most of California's cases was the Tahiti outbreak which involved primarily visitors to a resort on the island of Moorea. The majority of the cases imported to other states were infected in the Caribbean or Central America. Almost three quarters of the cases occurred from June through September, the months of the most extensive indigenous Aedes aegypti activity in the United States.

(Karl Kappus, Melinda Moore, Marjorie Pollack, Eugene Hurwitz, Lawrence Schonberger, and David Nelson)

REPORTS OF ARBOVIRAL INFECTION, UNITED STATES, 1978

STATE	SLE	CE	WEE	Other Encephalitis	Imported Dengue	
Alabama	9				2	
Alaska						
Arizona	2					
Arkansas		1				
California					31	
Colorado						
Connecticut		1			5	
Delaware						
Dist. of Col.						
Florida				5 EEE	4	
Georgia						
Hawaii						
Idaho	1					
Illinois		15			4	
Indiana	2	2			1	
Iowa		15				
Kansas						
Kentucky						
Louisiana					1	
Maine					1	
Maryland						
Massachusetts					3	
Michigan						
Minnesota		25	4		3	
Mississippi						
Missouri		1			3	
Montana						
Nebraska						
Nevada						
New Hampshire						
New Jersey					2	
New Mexico	2					
Upstate New York		23		1 powassan		
New York City					15	
North Carolina	1	2			1	
North Dakota						
Ohio	4	11			5	
Oklahoma						
Oregon						
Pennsylvania					3	
Rhode Island					2	
South Carolina						
South Dakota						
Tennessee	2	1				
Texas					2	
Utah						
Vermont						
Virginia					1	
Washington						
West Virginia						
Wisconsin		47				
Wyoming						
Totals	23	144	4		89	

REPORT FROM THE MEMPHIS AND SHELBY COUNTY HEALTH DEPARTMENT, MEMPHIS, TENNESSEE

Beginning in early April and ending in late October, 1978, the Insect Vector Control Division of the Memphis and Shelby County Health Department maintained continual daily surveillance of SLE/WEE arboviral activity in the area. Arboviral surveillance is accomplished with HI serological antibody analyses of avian blood serum, which includes: (a) wild-caught sparrows and (b) sentinel chicken flocks.

Table 1, attached, summarizes our serological data through early October, 1978.

Graph 1 correlates avian seroconversion data and mosquito population density for the 1978 surveillance season.

Wild bird seroconversion percentages generally remained well below the danger threshold of 4-5%, having risen above this level (7.3%) only once - in late June. This wild bird seropositive conversion rise coincided with a precipitous seropositive rise in sentinel chicken flocks, both increases having been preceeded by a sharp rise in mosquito population density. For the three-week period from June 26 through July 14, seropositive sentinel chicken conversions were: 5.6%, 14.3% and 5.5% respectively. Following this period, sentinel chicken seroconversion percentages generally dropped back to the normal level of 1-2%. This data suggests that we experienced only one period of intense SLE virus activity during the 1978 season. No confirmed cases of SLE were reported in Memphis in 1978.

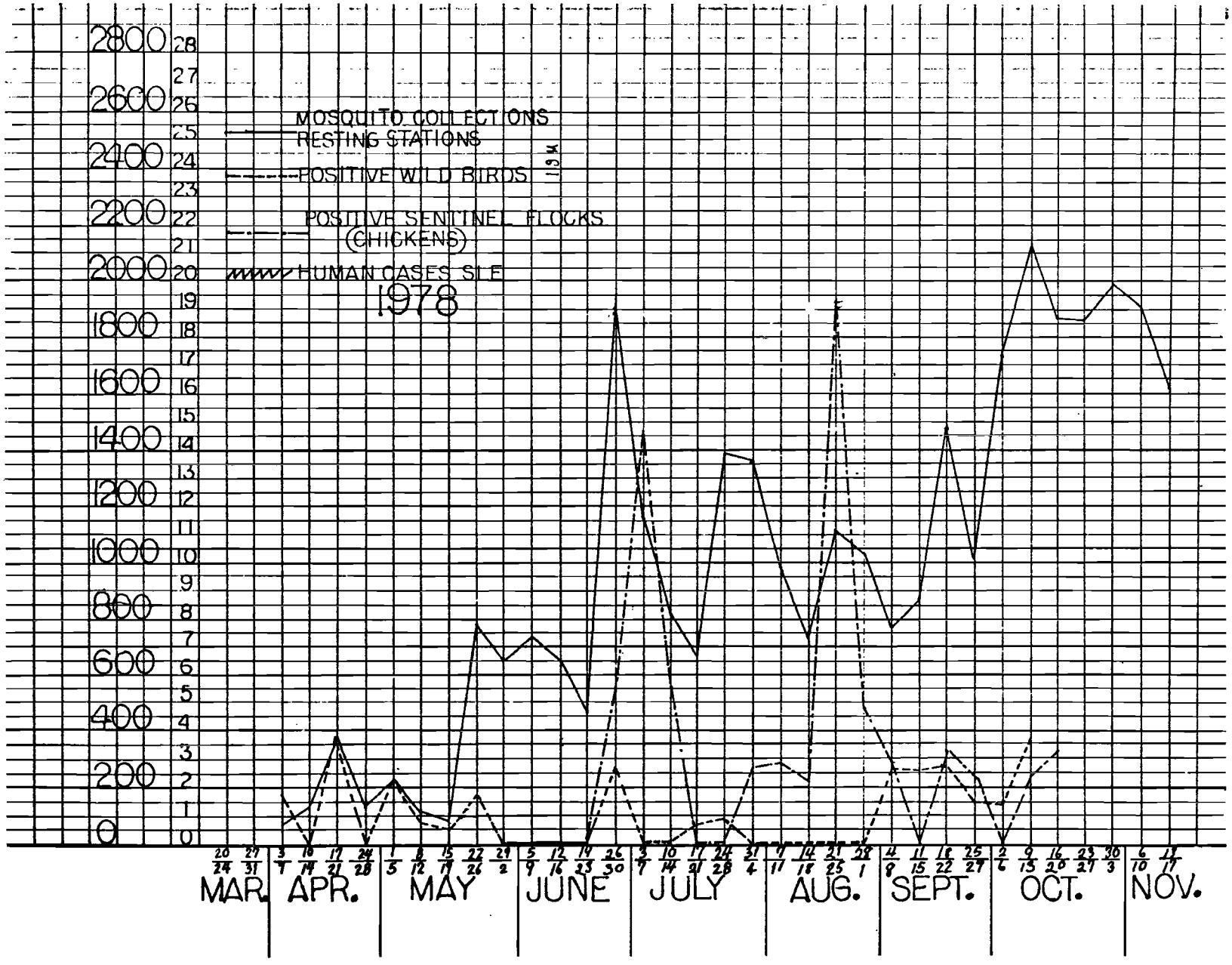
Insect (mosquito) vector control is closely integrated and coordinated with laboratory analyses and field surveillance. Consequently, the precipitous seropositive increases in wild birds and sentinel chickens resulted in the immediate application of intensified mosquito larviciding and adulticiding measures in the specific problem loci. This remedial control action may account for the fact that arboviral activity dropped back to the apparently normal level.

(James G. Hamm)

TABLE 1

Birds and Sentinel (Chickens) Tested for Hemagglutination—inhibition (HI) Antibodies to SLE Virus

Location	Number (percent) with SLE HI Titer ≥ 20/no. Tested						
	juv. / all ages	juv. / all ages	juv. / all ages	juv. / all ages	juv. / all ages	juv. / all ages	juv. / all ages
Memphis	Apr. 4-7	Apr. 10-14	Apr. 17-21	Apr. 24-28	May 1-5	May 8-12	May 15-19
wild birds	1/52 (2.0)	0/1 0/60	0/6 4/101 (4.0)	0/21 0/95	0/3 1/40 (2.5)	0/44 1/120 0.8	0/131 1/200 (0.5)
Sentinel chickens				0 12			
Memphis	May 22-26	May 29-June 2	June 5-9	June 12-16	June 19-23	June 26-30	July 3-7
wild birds	0/84 2/122 (1.6)	0/55 0/119	0/79 0/141	0/84 0/150	0/54 0/111	0/64 4/55 (7.3)	0/36 0/116
chickens		0/31	0/42	0/36	0/54	2/36 (5.6)	6/42 (14.3)
Memphis	July 10-14	July 17-21	July 24-28	July 31-Aug 4	Aug. 7-11	Aug. 14-18	Aug. 21-25
wild birds	0/28 0/114	0/47 1/95	0/53 0/59	0/24 0/31	0/81 0/56	0/69 0/78	0/84 1/48
chickens	2/36 (5.5)	0/42 (1.1)	0/36 (1.7)	1/40 (2.5)	1/36 (2.8)	1/41 (2.4)	7/35 (20%) (2.1)
Memphis	Aug. 28 Sept. 1	Sept. 4-8	Sept. 11-15	Sept. 18-22	Sept. 25-29	Oct. 2-6	Oct. 9-13
wild birds	———	0/16 2/70	0/2 2/82	0/5 2/7	0/3 1/61	0/1 1/54	1/14 1/40 (2.5)
chickens		1/34 (2.9)	0/40 (2.4)	1/31 (3.2) (2.8)	1/40 (2.5) (1.7)	0/32 (1.9)	(7.1) 1/41 (2.4)
Memphis	Oct. 16-20	Oct. 23-27					
wild birds	0/5 2/64 (3.1)	1/66					
chickens	1/32 (3.1)	(1.5)					


MOSQUITO COLLECTIONS
RESTING STATIONS
POSITIVE WILD BIRDS
POSITIVE SENTINEL FLOCKS (CHICKENS)
HUMAN CASES SLE
1978
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
MAR.
APR.
MAY
JUNE
JULY
AUG.
SEPT.
OCT.
NOV.

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

Arbovirus Surveillance in Pennsylvania, 1978

The Pennsylvania Departments of Health and Environmental Resources conducted an arbovirus surveillance program from June to October, 1978.

Sentinel flocks of four (4) chickens were placed at 49 locations throughout the state at the locations shown in Figure 1. There was at least one (1) sentinel flock in 21 of the 67 counties. The chickens were bled weekly and tested for hemagglutination - inhibition (HI) antibodies against St. Louis Encephalitis (SLE), Western Equine Encephalitis (WEE), Eastern Equine Encephalitis (EEE), California Encephalitis (CE), and Powassan (POW) viruses. There were no seroconversions in 14,570 HI tests performed.

In 1978, a total of 247 patients were tested serologically for evidence of infections with SLE, WEE, EEE, CE, and POW viruses. There were no confirmed cases.

Surveillance studies in prior years, using both serologic and isolation technics, have demonstrated the presence of all five (5) arboviruses in Pennsylvania.

(Bruce Kleger and Vern Pidcoe)

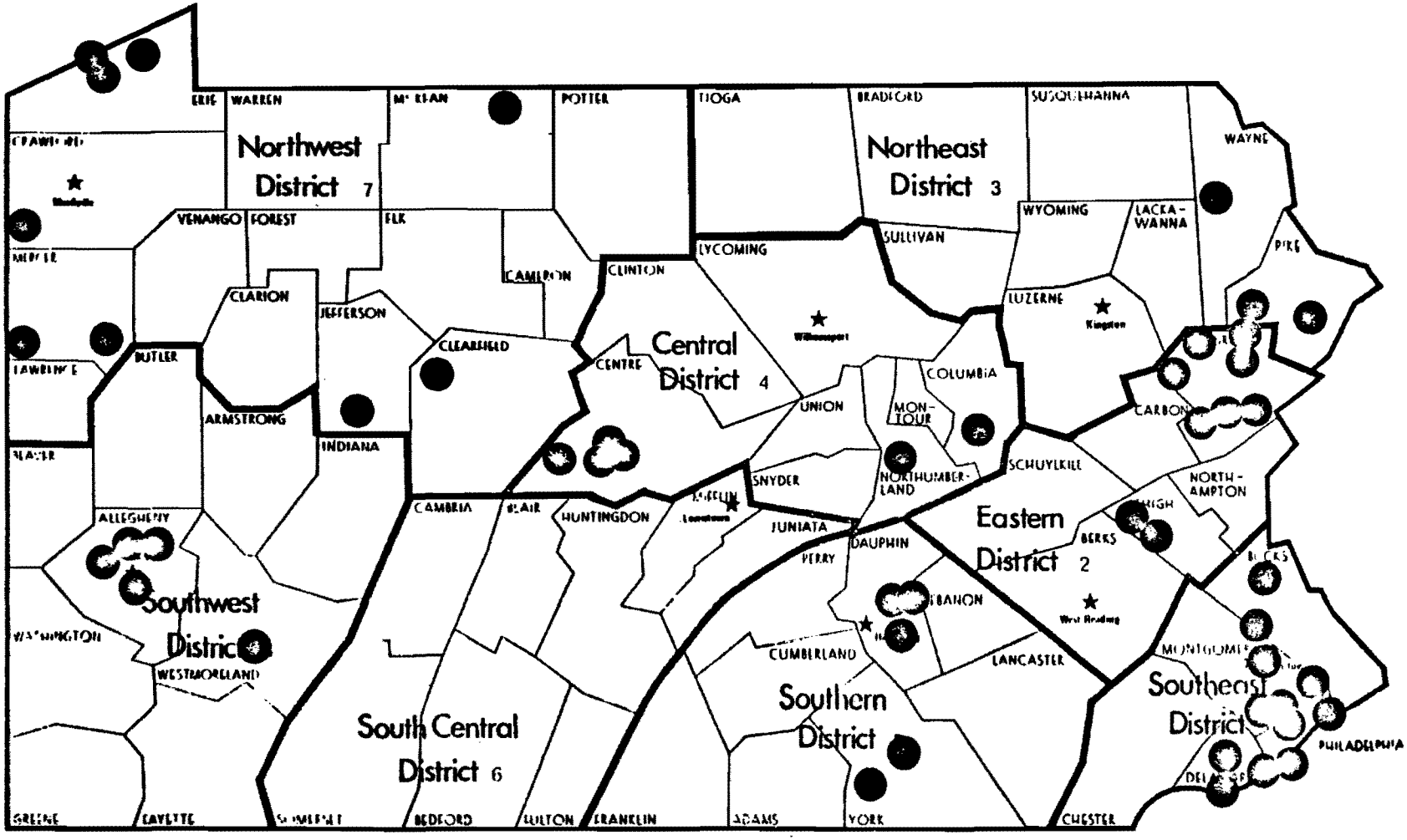


Fig. 1. Location of sentinel chicken flocks, Pennsylvania, 1978.

REPORT FROM THE STATE OF NEW JERSEY
DEPARTMENT OF HEALTH, TRENTON, NEW JERSEY

The following tables present findings concerning arboviruses in New Jersey, September and October, 1978, as part of a continuing arbovirus surveillance program by the New Jersey Department of Health. Findings through August, 1978, were presented in Information Exchange No. 35. No human cases were detected.

(B.F. Taylor)

Isolations from Arthropods in New Jersey, September 1978.

	Virus and No. of Strains			
Group	EE	WE	Isolated from	Collected in
A	2	9	C. melanura	Bass River & Route 9
A		4	C. melanura	Burlington County
A	5	6	C. melanura	Dennisville
A	2	10	C. melanura	New Gretna
A	1		A. quadrimaculatus	Woodbine
A	4	3	C. melanura	Woodbine
A	1		C. salinarius	Woodbine
Totals	15	32		

Isolation and/or Serology Animals in New Jersey, October 1978

Location	Animal Species	Date Specimen Collected	Isolation Data		Serology Data		
			Organs	Blood	HI	CF	Neut
Northfield	Equine	October 22, 1978	Brain	N.D.	B1 40 80 B2 320 640	N.D. N.D.	B1 0.7 B2 0.6

Isolations from Animals in New Jersey, September-October, 1978

Location	Animal Species	Date Specimen Collected	Isolation Data	
			Specimen	Virus
Woodbine	Pheasant	September 28, 1978	Egg Fluid	EE*
Forked River	Pheasant	October 23, 1978	Brain	EE

*Diagnosed by Dr. Tudor at Rutgers and confirmed in the Virology Laboratory

Isolations From Arthropods in New Jersey

October 1978

Virus and No. of Strains

Group	E.E.	WE	Isolated From	Collected in
A	1	1	C. melanura	Bass River
A	2	2	C. melanura	Burlington Co
A		1	C. pipiens	Burlington Co.
A	2		C. melanura	Dennisville
A	1		C. melanura	New Gretna
A	2	1	C. melanura	Woodbine
A		1	A. vexans	Woodbine
TOTALS	8	6		

REPORT FROM THE YALE ARBOVIRUS RESEARCH UNIT (YARU)
DEPARTMENT OF EPIDEMIOLOGY AND PUBLIC HEALTH
YALE UNIVERSITY, NEW HAVEN, CONNECTICUT 06510

Mokola virus, a rhabdovirus isolated from Nigerian shrews as well as man, has emerged as the serologic bridging agent among the viruses of the rabies serogroup. Although Mokola virus has never been isolated from insects, the establishment of persistent infections in Aedes albopictus cell culture implies that Mokola also may be the biological bridging agent between rabies and the vector associated rabies serogroup viruses such as Obodhiang and kotonkan. Studies were initiated at YARU to see if Mokola virus could replicate in and be transmitted by Aedes aegypti mosquitoes.

Mosquitoes were infected by intrathoracic inoculation with one of four strains of Mokola virus. The four strains with different passage histories used were: 1) Strain A had a history of mouse brain passage, 2) Strain B was adapted to Vero tissue culture after one passage through Aedes albopictus cell culture. 3) Strain C was recently adapted to Aedes albopictus tissue culture, 4) Strain D had a long history of passage in Aedes albopictus tissue culture and had established a persistent infection. At predetermined periods post-infection, mosquitoes were sacrificed, heads were severed, squashed, and examined for the presence of viral antigen by immunofluorescence. Portions of the bodies of certain mosquitoes were also examined for viral antigen by immunofluorescence. Remaining body materials were triturated and repassaged into noninfected mosquitoes (a mosquito infection passage-MIP). Initially this was done after two weeks extrinsic incubation, but the virus was found to develop slowly in the arthropod. Accordingly the incubation period was extended to one month. Attempts were also made to effect virus transmission to mice by bite.

Results: The Mokola strains B and D were found to replicate in Aedes aegypti (the former more efficiently) and were able to be passaged. Strains A and C were not. The results with strain A corroborate those obtained previously in this laboratory; mouse-brain-passaged virus was extremely inefficient in its ability to establish mosquito infection, and it could not be passaged.

The first series of virus passages utilizing strain B (Vero cells), involving 2-week incubation periods, yielded a few positive 2nd MIP mosquitoes but none of the 3rd MIP. When the incubation period was increased to 30 days, 5/5 of the 1st passage mosquitoes were found infected. Likewise many females of the 2nd MIP (Repeat) were found to be harboring virus. For example, at 2 weeks 3 females were positive by body squash but not by head squash. Two weeks later (32 days) one female was positive

by head squash and dissected salivary glands and thoracic ganglia contained antigen. At 38 days, one mosquito yielded positive head and body squashes. One week later (45-46 days), 1/2 females demonstrated FA antigen in head and body squashes. At 51-53 days, 6/8 females had positive head squashes and 4 of these 6 females on dissection yielded positive ventral nerve cords and thoracic ganglia. Thus this particular passage line of virus had been developing in mosquitoes for up to 83 days (1st MIP, 30 days and 2nd MIP (Repeat), 53 days).

Altogether 66 females were examined in this study with virus strain B and 36 (55%) were FA positive. Considering only those mosquito lines which gave a long history of virus incubation (30 days - at least at the time of passaging), 28/39 females (72%) were positive.

In one virus transmission attempt, 1st MIP mosquitoes after an incubation period of 34 days were allowed to feed. The recipient baby mice sickened or died in 4-5 days. Brains and organs, separately, of 3 bitten mice were passaged to fresh mice which in turn sickened, and from these an FA-positive heart smear (brain passage) and spleen smear (organ passage) were obtained. CF tests using FA-positive organs were negative; perhaps there was insufficient antigen present to yield a satisfactory CF antigen. Studies are continuing.

(T.H.G.Aitken, S.M.Buckley, B.J.Beaty, R.E.Shope)

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

Arbovirus Surveillance 1978

Humans. During 1978, 532 patients with signs of meningitis and/or encephalitis were examined for evidence of infection with Eastern equine encephalomyelitis (EEE), Western equine encephalomyelitis (WEE), St. Louis encephalitis (SLE), Powassan (POW) and California encephalitis (CAL) viruses. Laboratory findings indicated California encephalitis in 22 patients: serologic evidence of current infection was obtained in 2 of these cases and a presumptive diagnosis of recent infection was made in 20 patients. Most patients with California encephalitis had an onset of illness in July and August, but some cases occurred as early as June 8 and as late as October 31. Seventeen of the 22 patients resided in the eastern counties of New York State. CAL virus infection at an undetermined time was indicated by hemagglutination-inhibition and neutralization tests in 9 additional patients.

A POW virus infection was confirmed in an 8-year-old Rockland County boy with encephalitis and a history of prior travel in New England and southeastern New York.

Sentinels. In the Capital District area, where 7 of the CAL cases occurred, 14 domestic rabbits and 37 Syrian hamsters were exposed at 22 sentinel stations in 6 sites during the period of May 1 - October 12; one seroconversion to CAL virus was obtained in a sentinel hamster between August 3 and August 17.

Mosquitoes. A total of 3,873 pools of 193,344 mosquitoes collected in New York State during the past summer were tested for viruses. From 10 of 19 counties surveyed, 67 viral isolates were obtained as follows: EEE (2), WEE (3), CV (1) CAL (22) and FLA(39) (Table 1). Only one isolate of EEE was obtained from upstate New York where EEE virus activity has been detected annually since the initial equine outbreak in 1971. EEE and WEE viruses reappeared in Suffolk County (Long Island) for the first time since 1973. CV was detected in the northeastern part of the state for the first time following its initial isolation in 1975. In contrast to our 1977 surveillance which did not detect FLA virus, numerous FLA isolates were obtained in 1978 from central and southeastern New York.

Imported dengue fever 1977 and 1978

Following the 1977 outbreak of dengue fever in the Caribbean, 10 confirmed and 8 presumptive cases of dengue were diagnosed from New York State residents upon their return from affected areas. In addition, 11 immigrant Jamaican farm workers newly arrived in Ulster County were shown to have contracted the disease earlier (Serological tests for dengue fever were performed at the San Juan Laboratories, Bureau of Labs., Center for Disease Control, San Juan, Puerto Rico).

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

Laboratories for Virology
Division of Laboratories and Research
New York State Department of Health
Albany, New York 12201

Table 1

Arboviruses Isolated from Mosquitoes Collected in New York State
June - September 1978

Species	No. Tested		No. Isolated				
	Pools	Specimens	EEE	WEE	CV	CAL	FLA
Aedes aurifer	57	5,478					
Aedes canadensis	308	19,181					1
Aedes communis	175	6,103				4	
Aedes cantator	51	4,799				2	
Aedes cinereus	46	2,132				2	
Aedes stimulans	260	13,333				4	1
Aedes triseriatus	122	1,792					
Aedes vexans	456	26,272					
Mixed Aedes spp.	437	14,321				9	
Anopheles spp.	296	7,217			1		
Coquillettidia spp.	373	20,296				1	1
Culex spp.	670	46,946		1			26
Culiseta melanura	363	18,262	2	2			10
Other Culiseta spp.	242	6,907					
Other mosquitoes	17	305					
Total	3,873	193,344	2	3	1	22	39

REPORT FROM ARBOVIRUS SURVEILLANCE PROGRAM, DIVISION of LABORATORIES. ILLINOIS DEPARTMENT of PUBLIC HEALTH, Chicago, Illinois 60612

During 1978, primary emphasis of the Arbovirus Surveillance Program continued to be on vertebrate-vector studies of St. Louis encephalitis (SLE) virus but efforts in a recognized focus of LaCrosse (LAC) virus transmission were increased. Twenty-one species of birds were bled and tested for HI antibodies to SLE, Western Equine encephalitis (WEE), and Eastern Equine encephalitis (EEE). Antibodies to SLE virus were detected in 47 house sparrows (33 adults and 14 juveniles), 5 juvenile pigeons (rock doves), and 1 adult brown thrasher. One juvenile house sparrow had antibodies to WEE and one adult house sparrow had a 1:40 titer to EEE. A total of 4968 were tested; including 3373 juveniles and 1595 adults.

The 0.6 percent of the juvenile birds with antibodies to SLE marked the third consecutive year following the 1975 epidemic in which this value declined. Samples from 1976-1978 were collected from the same localities in similar seasonal patterns. No laboratory-diagnosed cases of SLE were found in Illinois during 1978.

Nestling and fledgling birds (946) predominantly house sparrows, were tested in primary duck embryo cell cultures (DECC) but no virus isolations were made. Mosquitoes (20,500 in 666 pools) were tested in suckling mice, DECC, or Vero cells. One, as yet unidentified virus, has come from a pool of 45 Culex spp collected on June 29. The above total included 5,104 laboratory-reared, Aedes triseriatus from Peoria County. The 123 pools of 2,443 females yielded five strains of a California (CAL) group virus while the 77 pools of 2661 male yielded a single strain of a CAL group virus.

Sixteen cases of CAL encephalitis were laboratory-diagnosed in Illinois during 1978 (Fig. 1). Ten resided in Peoria County, a persistent LAC focus. Serologic testing of equines was begun in the Division of Laboratories in cooperation with the Illinois Department of Agriculture this year. A total of 24 equines with "encephalitis" were tested but no arboviral etiology was identified.

Gary G. Clark and Harvey L. Pretula

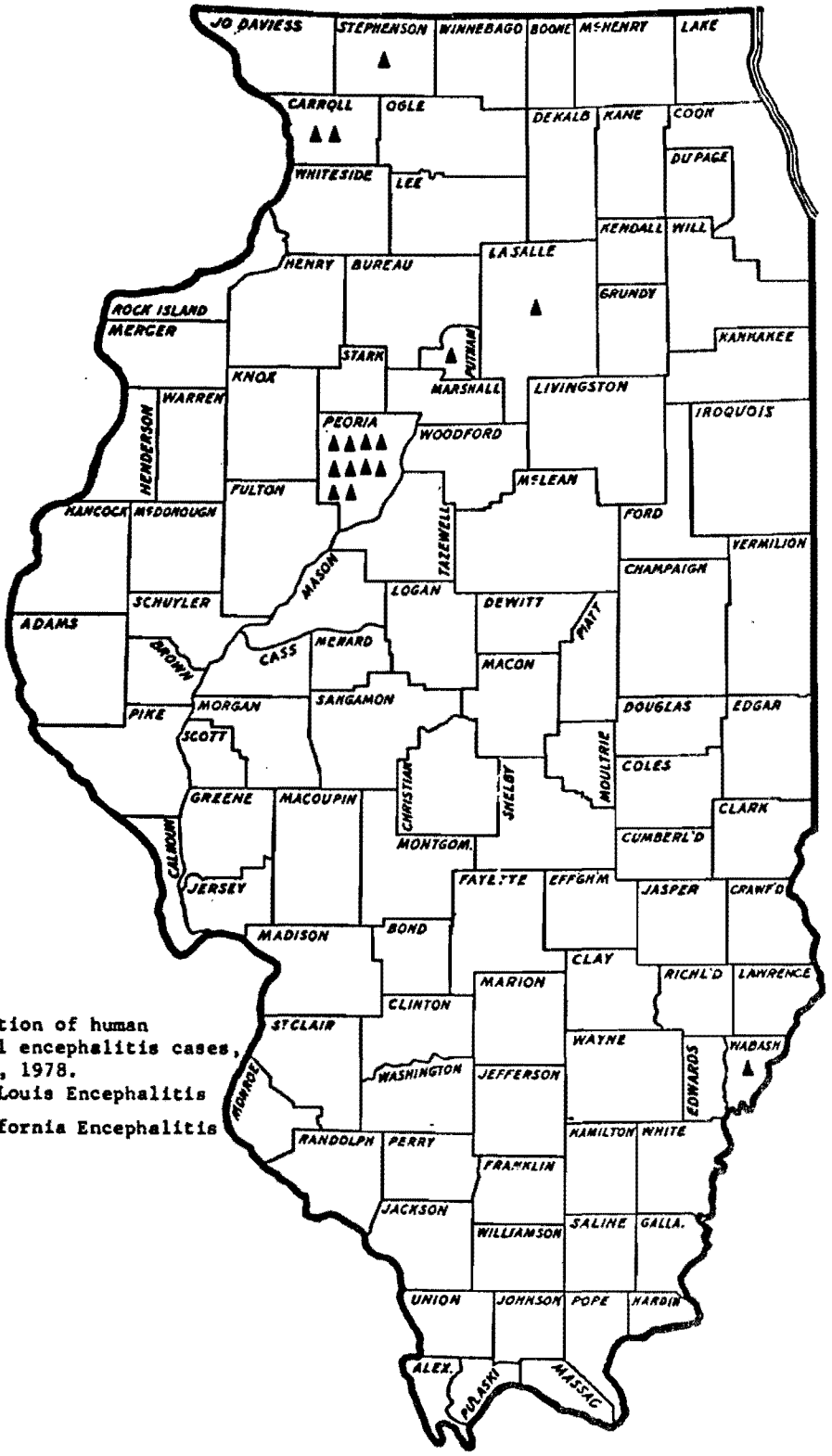


Fig. 1.
Distribution of human arboviral encephalitis cases, Illinois, 1978.
● St. Louis Encephalitis
▲ California Encephalitis

REPORT FROM THE MICROBIOLOGY SECTION,
THE HORMEL INSTITUTE, UNIVERSITY OF MINNESOTA
AUSTIN, MINNESOTA U.S.A.

The Use of Serum-Free Medium For Titration and Propagation of Dengue Viruses

An improved plaque assay for dengue virus was developed utilizing baby hamster kidney (BHK-21) cells initially grown in shaker culture. Eagle's minimal essential medium supplemented with 5% newborn calf serum was used for cell monolayer formation in 15 and 35 mm diameter wells (Linbro and Costar 24 and 6 well plastic plates). Cell monolayers were formed after 8 to 24 hours of incubation at 37°C depending on the pH and $NaHCO_3$ concentration in the medium. Sodium bicarbonate concentration of 1 mg/ml and pH 7.3-7.4 were optimal for fast cell monolayer formation. Dengue virus inoculation was carried 60 min at 37°C in a humidified CO_2 incubator. After infection, 0.5 and 2.5 ml of overlay medium was added to each of 15 and 35 mm wells, respectively. The overlay medium consisted of Eagle's minimal essential medium supplemented with 1% methylcellulose, penicillin (100 units/ml) and streptomycin (100 µg/ml). This medium was buffered with 10 mM 2-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) at pH 7.4-7.6. This serum-free overlay medium allowed for plaque formation after 3 days incubation of dengue type 2 virus and after 4 days for dengue types 1 and 4 viruses.

BHK-21 cells grown as suspended cultures in serum-free modified Waymouth 752/1 medium were used for propagation of dengue type 2 virus. Waymouth 752/1 medium was supplemented with 0.2% fatty acid-free bovine serum albumin and 20 mM $MgSO_4$. Medium was buffered with 20 mM HEPES at pH 7.6. Cells were infected with dengue type 2 virus using multiplicity of infection varying from 0.05 to 10 plaque forming units (PFU)/cell. After infection, 3×10^7 cells were suspended in 50 ml medium and cultivated at 37°C in a gyratory shaker. The maximum virus production occurred after 42-48 hours of incubation. The virus titer obtained for five different BHK-21 cell passages (278,286,297,396,398) varied from $10^{8.3}$ to $10^{8.8}$ PFU/ml.

The ability of BHK-21 cells grown in serum-free medium in shaker culture to support high yield of other types of dengue viruses is under investigation.

Barbara Malewicz and Howard M. Jenkin

REPORT FROM THE VECTOR-BORNE DISEASES DIVISION
CENTER FOR DISEASE CONTROL, FORT COLLINS, COLORADO

St. Louis Encephalitis Virus Transmission Following Multiple Feeding of *Culex pipiens pipiens* (Diptera: Culicidae) During a Single Gonotrophic Cycle.

Some mosquitoes may feed on a viremic host and ingest a small quantity of blood insufficient for initiating maturation of the oocytes, yet containing enough virus to infect the mosquito. After an appropriate extrinsic incubation period, such a mosquito could then become a potential vector while remaining nulliparous. Under experimental conditions, 3 of 31 (9.7%) *Culex pipiens pipiens* became infected with St. Louis encephalitis (SLE) virus after partial engorgement and without becoming gravid. In addition, SLE virus was transmitted from bird to bird by 2 *Cx. p. pipiens* during a single gonotrophic cycle.

If ingestion of an infective partial blood meal taken late in the summer or autumn were followed by successful hibernation, winter carryover of virus could be accomplished by nulliparous mosquitoes. Heretofore, barring the occurrence of gonotrophic dissociation or transovarial transmission of viruses, hibernating nulliparous mosquitoes have been discounted as possible sources of overwintering arboviruses.

(Carl J. Mitchell, G. Stephen Bowen, Thomas P. Monath, C. Bruce Cropp, and JoAnne Kerschner)

The Winter Biology of *Culex tarsalis* in Boulder County, Colorado

Three hundred and seventy-seven hibernating *Culex tarsalis* were collected from an abandoned gold mine tunnel near Jamestown, Colorado, on October 19, 1977, marked with a zinc sulfide dust, and released back into the mine. Recovery of only 8.7% of the marked population three to five months later indicates that mortality rates are high among *Cx. tarsalis* using this type of hibernaculum in Colorado. A total of 1,088 *Cx. tarsalis* were collected from surrounding mines from September 1977 through March 1978. The parity status of 606 specimens was: 2.8% parous, 89.9% nulliparous, and 7.3% undetermined. No virus was isolated from 905 *Cx. tarsalis* tested.

(Carl J. Mitchell)

Haemagglutinating Activity of Kaeng Khoi Virus

Routine assay of a sucrose-acetone extracted mouse brain antigen of Kaeng Khoi virus revealed that the antigen possessed hemagglutinating (HA) activity.

The antigen was tested for HA activity at pH 5.8 - 6.8 and at room temperature. Maximum HA (1:32 - 1:64) titers were obtained at pH 5.8 and 6.0, while no HA activity was detected above pH 6.4. In the CF test, the same antigen reacted with homologous mouse immune ascitic fluid to a titer of 512/512; and in the hemagglutination-inhibition (HI) test, HA was inhibited to a titer of 1:40 by homologous immune fluid.

A subsequent mouse brain antigen did not manifest HA activity even though it was sonicated during preparation and assayed in the presence of increased NaCl concentrations in the VAD's.

A hemagglutinin for Kaeng Khoi virus was prepared from Vero cell culture fluids concentrated 80-fold by PEG precipitation. Virus was plaque purified three times in Vero cell cultures, and the final plaque pick was used to prepare a virus seed in BHK-21 cell cultures. The BHK-21 virus seed was used as inoculum for Vero cell cultures. Maximum HA titers (1:128 - 1:256) were manifested at pH 6.3.

The Kaeng Khoi cell culture hemagglutinin was tested against NIH immune grouping fluids, and immune reagents to several individual viruses comprising the following:

Polyvalent groups ANA, ANB, TUR; Group BUN; Polyvalent Bwamba (BWA, PGA, Eret.-147, MOS, KAM): Group C; Group CAL; Group CAP; Polyvalent CON

(CHF-CON, HAZ, GAN, DUG, BHA); Group GMA; Polyvalent PAT (PAT, ZEG, SR, MIR, BER); Group PHL; Polyvalent QRF (QRF, JA, QYB, BDA, KSO, LJN, SIL); Group SIM; Polyvalent #1 (BAH, TETE, MTR, MTY, Eg An 1398-61, BEA); Polyvalent #2 (JUR, MNT, MARU-11079, GAM, BeAn 141106); Polyvalent #3 (KOO, WON, BAK, KET, MAP, MPK, TRU); Polyvalent #4 (NYA, UUK, GA, THO); Polyvalent #5 (HUG, SOL, SAW, MAT, LS); Polyvalent #6 (MCO, CHO, TIM, PAC); Polyvalent #9 (NAV, TNT, ARU, PCA); Polyvalent #10 (UPO, DGK, WAN, DHO). Polyvalent #12 (OKO, OLI, WIT, BIA, TAT); immune reagent to Sunday Canyon virus; to Guaratuba; and two Kaeng Khoi virus immune reagents. The hemagglutinin was inhibited only by the homologous immune fluids, to titer of 1:40 and 1:320 respectively.

Kaeng Khoi virus was not neutralized at a 1:20 dilution when it was reacted in 90% plaque reduction neutralization tests (PRNT) against immune reagents for viruses of the Bunyamwera group. Homologous Kaeng Khoi immune fluid neutralized the virus to a titer of 1:80.

Kaeng Khoi virus still remains ungrouped, though provisionally placed in the Bunyamwera Supergroup, unassigned (SBU).

(N. Karabatsos, James H. Mathews, and Ann Hunt
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A New Arbovirus Related to Gamboa Virus

Field studies on arboviruses in Ecuador and Argentina have resulted in the isolation of 28 virus strains from Aedeomyia squamipennis between 1975 and 1977. In complement-fixation tests these isolates reacted with hyperimmune mouse ascitic fluids to prototype Gamboa virus (MARU 10962). One of the strains from Ecuador (75V-2374) was used in cross-neutralization tests to define identity with prototype Gamboa virus (Table 1). The results indicated that 75V-2374 was a new virus related to but distinct from Gamboa virus. The name Abras virus is proposed, and the establishment of a Gamboa serogroup of Bunyaviruses is suggested.

The remaining field isolates were tested by neutralization against Gamboa and Abras antisera. Fourteen strains from Ae. squamipennis collected in the littoral zone of Ecuador in 1975 were most closely related to prototype Gamboa virus. Five strains from the same areas collected in 1974 and 1975 were identified as Abras virus.

Two strains isolated from Ae. squamipennis collected near Santa Fe, Argentina in the spring of 1977 (Nov-Dec) were typed as Abras virus.

Six strains remain untyped.

A serologic survey of birds and mammals from Santa Fe Province was performed, using the plaque-reduction N test and abras virus (Argentina strain 78V-2441). Results, shown in Table 2, indicate high antibody prevalences in wild and domestic birds and low or absent evidence for infection of mammals. These findings are consistent with the known aviaphilia of Aedeomyia squamipennis and suggest that birds

may be involved in the transmission cycle. As reported by Galindo et al (Arbo Info Exch No. 35), Gamboa virus is transovarially transmitted in Ae. squamipennis. Circumstantial evidence suggests that this is probably also the case for Abras virus, since a high field infection rate in Ae. squamipennis (8 per 1,000 mosquitoes) was found in the springtime in Argentina. (T.P. Monath, C.H. Calisher, D.B. Francy, J.S. Lazuick, C.B. Cropp, C.J. Mitchell, G.S. Bowen, E. Gutierrez, M. Sabattini)

TABLE 1. Cross-neutralization test results with Gamboa and 75V-2374 viruses.

	Antisera	
Virus (strain)	Gamboa	75V-2374
Gamboa (MARU 10962)	≥128	<4
75V-2374	16	32

TABLE 2. Plaque reduction N test serosurvey of wild mammals and birds for antibodies to Abras virus (78V-2441), Santa Fe Province, Argentina

	Family or Species	No. Pos./No. Tested (%)
Avians	Furnaridae	9/87 (10.3)
	Fringillidae	2/25 (8.0)
	Ploceidae	1/120 (0.8)
	Columbidae	4/15 (26.7)
	Phytotomidae	1/12 (8.3)
	Other wild birds (8 Families)	0/52
	Domestic birds	45/117 (38.5)
	Subtotal	63/428 (14.7)
Rodents	Cavia aperea pamparum	1/18 (5.6)
	Akodon obscurus	1/14 (7.1)
	Other (8 species)	1/44 (2.3)
	Subtotal	3/76 (3.9)
Marsupials	Lutreolina crassicaudata	0/12
	Other (2 species)	0/6
	Subtotal	0/18
Lagomorphs	Domestic rabbit	0/23

Comparisons of vector-unassociated flaviviruses

The 59 recognized flaviviruses are classified as such taxonomically but they are a biologically diverse group of antigenically related agents. Twenty-seven are mosquito-borne and 14 tick-borne but for the remaining 18 no arthropod vector has been demonstrated in nature and their method(s) of transmission is unknown. If these viruses are not transmitted by arthropods then they represent a distinct biological subgroup related, of course, antigencially but more importantly, perhaps by their mode of transmission. They may be extremely primitive (stem viruses) or highly adapted viruses, transmitted mechanically through urine, saliva or other excretions.

Experimental transovarial transmission of Koutango virus in Aedes aegypti by Coz et al. (C.R. Acad. Sci. Paris, 283:109-110, 1976) and serologic evidence that Negishi is antigenically most close to the tick-borne complex implies that an accumulation of biologic and serologic information would be helpful in determining which of these flaviviruses may actually be mechanically transmitted.

As a first step in determining the validity of such an hypothesis we have begun to characterize a number of vector-unassociated flaviviruses (Table 1). Seed viruses, prepared in suckling mice were titrated in a variety of hosts (Table 2). Interestingly, only 18 of 21 produced plaques in duck embryo cell cultures, while 15 of 21 produced plaques in Vero cells and 18 of 21 produced plaques in $LLCMK_2$ cells. Whether or not ability to produce plaques in mammalian

but not avian cells is an indicator of a mammalian phylogenesis remains unanswered. All but Carey Island and (MA-387-72) hemagglutinated goose erythrocytes, 11 had an optimum titer at pH 5.9-6.0, 4 at 6.3-6.4 and 4 including Koutango, at 6.5-6.8.

Next we examined four strains of Modoc virus by complement-fixation: prototype M-544 and isolates DPG-1333, DPG-1337 and PM-160, all from Peromyscus maniculatus. The results shown in Table 3 indicate a modicum of differences between the prototype and both DPG-1333 and DPG-1337. When complement-fixation (Table 4) and neutralization (Table 5) test results with 8 vector unassociated flaviviruses were examined we observed a complex of viruses (Jutiapa, Modoc, Cowbone Ridge and isolates MA-387-72 and 71V-1251 (shown to differ in other tests).

Additional serologic and other tests are in progress.

I. V. Wesley and C. H. Calisher

Table 1. Viruses used in studies of vector unassociated flaviviruses by strain, source, date collected and origin.

Virus	Abbr.	Strain	Source	Collected	Country of Origin	Obtained from
Apoi	APOI	Original	Apodemus speciosus	October 9, 1954	Japan	N. Karabatsos
Aroa	AROA	VeNA 1809	(Sentinel hamster)	?, 1972	Venezuela	R. Shope
Batu Cave	BC	P71-1030	Eonycteris spelaea	July 2, 1971	Malaysia	R. Shope
Carey Island	CI	P70-1215	Cynopterus brachyotis	January 15, 1970	Malaysia	R. Shope
Cowbone Ridge	CR	W-10986	Sigmodon hispidus	January 10, 1965	U.S.A.	N. Karabatsos
Dakar Bat	DB	IPD/A249	Vespertilionides-scotophilus	September 4, 1962	Senegal	N. Karabatsos
Entebbe Bat	ENT	IL-30	Tadarida limbata	July 4, 1957	Uganda	N. Karabatsos
Israel Turkey Meningo.	IT	Original	Meleagris gallopavo	September, 1959	Israel	R. Shope
Jutiapa	JUT	JG-128	Sigmodon hispidus	August 8, 1969	Guatemala	N. Karabatsos
Koutango	KOU	DakAnD 5443	Tatera kempi	April 24, 1968	Senegal	R. Shope
Modoc	MOD	M544	Peromyscus maniculatus	July 9, 1958	U.S.A.	N. Karabatsos
Mont. Myot. Leukoenc.	MML	40649	Myotis lucifugus	July 29, 1958	U.S.A.	N. Karabatsos
Negishi	NEG	Original	human	August, 1948	Japan	N. Karabatsos
Phnom Penh Bat	PPB	A38/69	Cynopterus brachyotis	June 11, 1969	Cambodia	N. Karabatsos
Rio Bravo	RB	M64	Tadarida brasiliensis	October 1, 1954	U.S.A.	N. Karabatsos
Saboya	SAB	DakAnD4600	Tatera kempi	February 22, 1968	Senegal	R. Shope
Sokuluk	SOK	LEIV-400K	Vespertilio pipistrellus	May 8, 1970	U.S.S.R.	R. Shope
Tamana Bat	TB	Tr127154	Pteronotus parnelli	September 12, 1973	Trinidad	R. Shope
		78TWM106	Peromyscus leucopus	August 28, 1978	U.S.A.	R. McLean
		MA-387-72	Sigmodon hispidus	July 16, 1972	U.S.A.	A. Lewis
		71V-1251	Sigmodon hispidus	July 30, 1971	U.S.A.	D. B. Francy
		DPG-1333	Peromyscus maniculatus	June 4, 1963	U.S.A.	K. Smart
		DPG-1337	Peromyscus maniculatus	June 4, 1963	U.S.A.	K. Smart
		PM-160	Peromyscus maniculatus	?	Canada	R. Zarnke

Table 2. Results of titrations of certain flaviviruses in suckling mice and Vero, $LLCMK_2$ and primary duck embryo cells.

Virus	SM	Titer[a] in: Vero	$LLCMK_2$	DE
APOI	7.4 (5)[b]	6.0 (8)	6.3 (6)	<3.0
AROA	9.0 (8)	5.6 (9)	6.6 (9)	7.0 (6)
BC	9.0 (7)	8.0 (7)	8.5 (6)	<3.0
CI	9.2 (7)	4.0 (8)	4.0 (6)	<3.0
CR	5.2 (8)	5.1 (9)	4.0 (12)	2.7
DB	5.3 (7)	<3.0	<3.0	4.0 (6)
ENT	7.4 (4)	7.0 (6)	<3.0	<3.0
IT	7.1 (4)	8.0 (7)	6.9 (9)	7.3 (6)
JUT	8.7 (9)	<3.0	7.2 (6)	<2.7
KOU	9.7 (4)	8.3 (6)	8.5 (6)	8.0 (5)
MOD	9.4 (7)	8.3 (8)	8.2 (7)	7.3 (6)
MML	9.3 (8)	6.9 (8)	7.8 (7)	7.0 (6)
PPB	9.2 (8)	5.2 (8)	8.7 (6)	<3.0
RB	10.2 (4)	<1.7	9.4 (10)	7.9 (6)
SAB	7.3 (5)	7.3 (6)	<3.0	5.3 (6)
SOK	8.9 (5)	7.0 (7)	7.0 (6)	<3.0
TB	7.1 (9)	<3.0	7.3 (6)	<3.0
(78TWM-106)	8.8 (8)	<3.0	6.3 (8)	<3.0
(MA-387-72)	8.7 (9)	<2.7	5.8 (11)	<2.7
(71V-1251)	$\geq$6.2 (7)	8.3 (9)	6.0 (11)	<3.0
(PM-160)	7.5 (7)	8.7 (9)	9.1 (9)	<3.0

[a] $\log_{10}$SMicLD$_{50}$ or PFU/ml

[b] Numbers in parentheses indicate average survival time in mice or time to appearance of plaques in cell cultures.

Table 3. Cross box complement-fixation tests with Modoc virus (M-544) and three isolates from Peromyscus sp.

Antigen	Titer[a] with MAF to virus: Modoc	(PM-160)	(DPG-1333)	(DPG-1337)
Modoc	≥1024	512	256	128
(PM-160)	512	512	512	128
(DPG-1333)	512	128	128	64
(DPG-1337)	≥1024	256	256	512

[a]Results given as reciprocal of titer of MAF at optimal antigen dilution.

Table 4. Results of cross box complement fixation tests with 8 flaviviruses

Antigen	Antibody to: JUT	MA-387-72	71V-1251	MOD	CR	MML	PPB	RB
JUTIAPA	≥1024	≥1024	≥1024		8	8	16	
(MA-387-72)	128	≥1024	≥1024	8	8	8	16	8
(71V-1251)	256	≥1024	≥1024	8	8	8	8	8
MODOC	32	≥1024	≥1024	64	8		8	
COWBONE RIDGE	32	256	256	16	256	8	8	8
MONT. MYOT. LEUKOENC.		16	16			64	32	
PHNOM PENH BAT	8	16	32			16	16	8
RIO BRAVO	8	64	128				8	128

Blank = <8

Table 5. Results of cross neutralization tests in cell cultures with 8 flaviviruses

Virus	JUT	MA-387-72	71V-1251	MOD	CR	MML	PPB	RB
JUTIAPA	≥640	≥640	≥640					
(MA-387-72)		≥640	≥640					
(71V-1251)	≥640	≥640	≥640		40	20	160	
MODOC	40	40	≥640	40	160			
COWBONE RIDGE	160	≥640	≥640		≥640		20	
MONT. MYOT. LEUKOENC.						160		
PHNOM PENH BAT							160	
RIO BRAVO								40

Blank = <20

Ixodid tick viruses. Continuing collaboration with WHO/University of Wisconsin influenza investigators (Dr. Bernard Easterday, project leader) has resulted in the recognition of three viruses from seabird parasites, Ixodes uriae (Ixodidae), collected on St. Paul Island, Alaska. Two of these viruses were cloned by plaque isolation and identified by IF, CF and NT tests. One is an Orbivirus of the Kemerovo group previously found by us in blood of a seabird on St. Paul Island. It is closely related to and possibly indistinguishable from Okhotskiy virus described by Soviet workers from ticks collected on islands and coastal areas of the Sea of Okhotsk. Another is a member of the family Bunyaviridae (Uukuniemi group) and is distinct from five other members of the UUK group by the above serological tests. The third agent represented in these ticks, a Sakhalin group virus (unclassified), could not be isolated, but its presence was recognized by CF reactions to inoculated tissue cultures.

Argasid tick viruses. A Quaranfil group agent, which we isolated in 1976 from Ornithodoros capensis ticks (Argasidae) collected in a Cape Cormorant nesting area of Walvis Bay, Southwest Africa, has been identified as Johnston Atoll virus. This virus was first reported from Central Pacific islands of the Hawaiian Chain, and has subsequently been found in Australia. Its occurrence in SW Africa considerably extends its known range.

In 1977 (Arth. Virus Info. Exch., No. 32), we noted the existence of Soldado virus (Hughes group) in a south Texas, U.S.A. population of Ornithodoros capensis. Last year we returned to the locality of collection (Aransas Bay National Wildlife Refuge) and, with the helpful assistance of Mr. Kirke King, Research Biologist, Patuxent Wildlife Research Center, Gulf Coast Field Station, Victoria, Texas, collected 815 O. capensis from vacated nests of reddish egrets on Long Reef Island. Of 120 male and 120 female ticks that were tested individually in suckling mice and tissue cultures, 13 males and 11 females yielded viral isolates that proved to be Soldado virus.

Soldado virus is typical of many Hughes group agents in its low pathogenicity for mice and tissue cultures. In the above tests, none of the isolates were obtained through inoculation of newborn mice, only two were obtained in Vero cells, but 22 were made in an amphibian cell line (Xenopus laevis), kindly provided by Dr. Mary Pudney of the London School of Hygiene, London, England. These toad cells also show promise in the assay, by plaque enumeration, of certain other arboviruses. We tested its ability to form plaques in response to infection with 105 arboviruses, mostly tickborne and of various serogroups. It is the best system to date for plaque assay of Sakhalin group viruses and possesses advantages over Vero cells for the assay of some Uukuniemi group agents and of Hazara virus.

In collaboration with Dr. Emmett R. Easton, South Dakota State University, Brookings, we isolated nine strains of viruses from Cliff Swallow parasites, Argas cooleyi, taken in nesting habitats of South Dakota. Two viruses previously described by us from Texas, New Mexico, and Colorado were recognized in this material: Sapphire II, an unclassified agent of the Hughes serogroup, and Sixgun City, an Orbivirus of the Kemerovo group. A large series of cimicids (bat-bugs) collected from the same habitat were also tested for viruses, with negative results.

Viruses in arthropod cells. An alphavirus we earlier found to be persistently infecting some strains of cultured mosquito cells was studied further. This virus, originally latent in these cells, can be expressed by stress or by subinoculation into uninfected cells. Infected cells significantly resist challenge of related viruses, Chikungunya (CHIK) and O'nyong-nyong, but not of other unrelated viruses. This year we found that mosquito cells that had recovered from infection with the alphavirus were markedly resistant to challenge with CHIK virus, indicating that the former agent may have reverted to latency. This suggests the possibility that proviral DNA had become incorporated into the cellular genome. Alternatively, it is possible that the alphavirus exists as defective interfering particles.

Attenuation of Colorado tick fever (CTF) virus after prolonged arthropod cell passage is under study. Previously, we observed that prolonged growth in mosquito cells of the Florio strain of CTF virus (a more virulent, small plaque strain) lessened its virulence and increased the predominant size of its plaques. In these respects it resembles wild strains. It was suggested that selection of larger, less virulent plaque-variants might have been effected in the arthropod cells. This year, in another experiment employing cloned, small plaque Florio virus, we again demonstrated loss of virulence after prolonged growth in Aedes cells. However, plaque size was not altered. This suggests that a potential for attenuation is present in the virion regardless of plaque morphology and that some other mechanism, such as temperature sensitive mutation or variation, may be operative.

(C. E. Yunker, C. M. Clifford, J. E. Keirans, and L. A. Thomas)

REPORT FROM THE VIRAL AND RICKETTSIAL DISEASE LABORATORY SECTION, CALIFORNIA DEPARTMENT OF HEALTH SERVICES, BERKELEY, CA

During 1978 at least 367 patients were tested for western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE), as well as for the more likely causes of encephalitis (mumps, herpes, enteroviruses, etc.). This surveillance effort is increasingly being participated in by the larger local health department laboratories which have developed viral diagnostic capabilities. A large proportion of the sera from cases not yet identified as to etiology will subsequently be tested by Dr. W. C. Reeves' research group (14th year of this study) to search for possible relationship of encephalitis to mosquito-borne viruses other than WEE and SLE viruses. There were 10 human brain samples and 1 cerebrospinal fluid sample from fatal cases of encephalitis which were tested in suckling mice during 1978, but none yielded arboviruses.

Only 1 case of SLE was detected in 1978: a 29 year old man from La Mesa, San Diego County, who was most likely infected by mosquito bites at a country club golf course in nearby Lakeside the weekend of September 30/October 1. A prior trip to Mexicali and El Centro 2 weeks earlier was beyond the maximum incubation period. Onset of illness was October 20, and fever, progressive headache, vomiting, stiff neck and some disorientation characterized the disease course. He was hospitalized from October 24 until November 1, and recovered without apparent sequelae. Serum samples taken October 24 and November 7 showed rising SLE antibody titers, confirming the diagnosis: complement-fixing (CF) antibody titers were 1:4 and 1:16 in the San Diego Laboratory (<1:8 and 1:32 in the state Viral and Rickettsial Disease Laboratory); and indirect fluorescent antibody (IFA) titers were 1:32 and 1:128, respectively. Hemagglutination-inhibition and plaque-reduction neutralization titers are pending. Mosquito collections made at sites nearby the apparent site of this exposure, during the summer period prior to and subsequent to the case occurrence, did not yield viruses. However, possible vector species (Culex tarsalis, Culex pipiens) were present.

There were 35 suspect cases of encephalitis in equines reported to the Department from 18 counties in the state, and 12 were found to be positive or presumptive-positive WEE cases by serologic tests. This is a significant increase in the total number and the percent positive over the findings of the past several years (only 6 confirmed cases from 1972-1977), and correlates with the marked increase in WEE virus activity during 1978. Of 10 equine brain samples from fatal cases of suspected encephalitis which were tested in suckling mice, all were negative for arboviruses. In addition, 7 brain samples from squirrels dying of suspected encephalitis were tested, but no viruses were isolated. Such squirrel brains have sometimes yielded WEE virus in past years.

A total of 1,798 mosquito pools (including 77,914 mosquitoes) were collected and tested in suckling mice during the year. There were 187 viruses isolated, including 87 WEE, 39 SLE, 39 Turlock, and 22 Hart Park. All isolates were from Culex tarsalis except 4 WEE and 2 SLE from Culex erythrothorax, 2 WEE and 1 Turlock from Culex pipiens, and 1 WEE from Aedes nigromaculis. The large reservoir of WEE and SLE viruses, the increase in equine cases of WEE, and the prospects for abundant water and potential mosquito breeding sites during the 1979 summer, indicate the continuing need for surveillance and control efforts to prevent involvement of an increasingly susceptible human population.

The other mosquito-borne virus disease of interest this year was dengue, which was proven or presumptive positive in at least 37 cases, the largest number ever recorded in modern times in the state. These included travelers returning from Tahiti (28), Puerto Rico (3), Colombia (1), El Salvador (2), Djakarta, Indonesia (1), the Seychelles (Indian Ocean) (1), and SE Asia (1). In the absence of Aedes aegypti or other suitable vectors in California, this disease is of less interest to vector control agencies than another "exotic" disease -- malaria -- which can be spread by native Anopheles species. At least 226 cases of imported malaria were recorded during 1978.

There were only 6 cases of Colorado tick fever, a low number indicating that the virus was at a low ebb in its natural tick-rodent cycle, or perhaps simply less interest by physicians in having their clinical suspicion verified by specific laboratory tests.

(R. W. Emmons)

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

California Group Activity in Ontario, 1978

1. Indicator Rabbit Studies

On May 26, 1978, sixteen New Zealand white rabbits were placed at four sites in Essex county (Southern Ontario) with four rabbits per cage. These rabbits were bled weekly and tested for antibodies to the following arboviruses - eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, Powassan and four California group viruses - Snowshoe Hare (SSH), Jamestown Canyon (JC), LaCrosse (LAC) and Trivittatus (TVT). Rabbits were maintained in the field until October 10th. When seroconversions were obtained, the positive rabbits were brought in from the field and replaced so that four sentinel rabbits were continuously maintained at each site. This study was done in collaboration with Dr. G.A. Surgeoner of the University of Guelph.

Seroconversions to California group antigens were obtained in five rabbits, all of which were maintained at the same site in Ojibway. Two seroconversions occurred in June (June 14 and 28), two in July (both on July 26) and one on August 16. Complement fixation serology indicated that all five rabbits were infected with the SSH serotype (Table 1). No seroconversions were obtained in sentinel rabbits at the three other sites - Woodslea, St. Clair Beach and Amherstburg.

An intensive field program to study California group virus activity at the Ojibway site is planned for 1979.

2. Human Infections

a) Serologically Diagnosed Case

In late June, a 30-year-old male from Bracebridge, Ontario was admitted to Toronto General Hospital with aseptic meningitis. Sera taken 6 and 10 days post onset showed two-fold increases in titer to California group antigens by hemagglutination inhibition and complement fixation. A third serum taken 16 days post onset showed no further change in antibody titers by these serological tests (Table 2). However, tests conducted by Dr. C.H. Calisher

of the Center for Disease Control, Fort Collins showed a diagnostic rise by neutralization to California group viruses with highest titers to SSH virus.

b) Infection of Summer Student

On August 28, our summer student employed doing field work (bleeding indicator rabbits, trapping small mammals, collecting mosquito larvae) had an onset of symptoms including fever, headache, stiff neck and lethargy. Serology performed on this student showed antibodies to California group viruses with highest titers to SSH (Table 2). No diagnostic changes in titer were obtained to indicate current infection but high neutralization titers were found to SSH virus.

(H. Artsob, L. Spence and C. Th'ng)

Table 1

Complement Fixation Reactions of Sentinel Rabbits to California Group Antigens

RABBIT NUMBER	CALIFORNIA GROUP ANTIGEN	DATE OF BLEEDING (1978) JUNE 14	JULY 6	JULY 26	AUGUST 11	AUGUST 16	AUGUST 23	SEPT. 1	SEPT. 27	OCT. 18
3-2	SSH	<4[1]	64	64						
	JC	<4	16	16						
	LAC	<4	16	16						
	TVT	<4	ac[2]	ac						
3-3	SSH		128		128	64		32	32	16
	JC		ac		ac	ac		ac	4	ac
	LAC		ac		16	32		16	16	ac
	TVT		<4		<4	ac		<4	<4	<4
3-4	SSH	32	32		32					
	JC	<4	<4		ac					
	LAC	<4	<4		<4					
	TVT	<4	<4		<4					
3-5	SSH		128		64	64		64	32	32
	JC		<4		ac	<4		8	8	8
	LAC		ac		8	8		8	8	8
	TVT		<4		ac	<4		<4	<4	<4
3-6	SSH				<4	32	128			
	JC				<4	<4	8			
	LAC				<4	<4	ac			
	TVT				<4	<4	<4			

1. Reciprocal of complement fixation titer.
2. Anticomplementary.

Table 2

Human Infections due to California Group Virus(es) in Ontario, 1978

Date Blood Taken	Serological Test								
	Hemagglutination Inhibition			Complement Fixation			Neutralization[1]		
	SSH	JC	LAC	SSH	JC	LAC	SSH	JC	LAC
a) Serologically Diagnosed Case									
26/6/78	160[2]	N.T.[3]	160	16	N.T.	< 4	320	160	80
30/6/78	320	N.T.	320	32	N.T.	< 4	≥ 640	320	320
6/7/78	320	N.T.	320	32	N.T.	< 4	≥ 640	320	320
b) Infection of Summer Student									
29/8/78	40	40	80	4	<4	<4	≥640	160	320
31/8/78	40	40	80	N.T.	N.T.	N.T.	≥640	160	320
13/8/78	40	20	80	4	<4	<4	≥640	160	160

1. Neutralization tests were kindly performed by Dr. C.H. Calisher, CDC, Fort Collins.

2. Reciprocal of antibody titer.

3. Not Tested.

REPORT FROM THE DIVISION OF LABORATORIES
MINISTRY OF HEALTH, VANCOUVER
BRITISH COLUMBIA, CANADA

Through the calendar year 1978 no human cases of arthropod-borne infection have been identified in British Columbia. A survey of veterinarians in the area has also failed to reveal evidence of arboviral infections in equines or other animal species.

(G.D. Kettyls)

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY,
UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, B.C., V6T 1W5, CANADA

From 20,305 unengorged female mosquitoes of 4 species collected in the western Canadian Arctic between latitudes 60 and 69°N from 5 June through 24 July 1978, 9 strains of California encephalitis (CE) virus (snowshoe hare subtype) were isolated from Aedes communis, 4 strains of CE virus were isolated from Culiseta inornata and 3 strains of Northway (NOR) virus were isolated from Cs. inornata. Minimum field infection rates (MFIR) for CE virus in A. communis ranged from 1:159 at Annie Lake (60½°N, 135°W) through 1:168 at Marsh Lake (61°N, 134°W), 1:659 and 1:780 along the Dempster Highway (65-66°N, 138°W) to 1:3003 at Inuvik, N.W.T. (69°N, 135°W), whilst MFIR for CE in Cs. inornata were 1:73 to 1:112 along the Dempster Highway. The MFIR for NOR virus in Cs. inornata were 1:267 at Carcross (60°N, 135°W) and 1:1322 at Marsh Lake. For the first time, CE and NOR viruses were isolated during the same summer at Marsh Lake, Y.T.

Wild-caught Cs. inornata and A. communis have been held for periods exceeding 6 months at 4°C following intrathoracic injection with 300 or 30 PFU of CE virus (75-L-10 strain) or NOR virus (76-Y-330 strain). For Cs. inornata, infectious CE virus was first detected in salivary glands at 27 days and again after 124, 166 and 218 days when titers attained 3.0 log PFU per gland. For A. communis, CE virus was detected in salivary glands after 97 and 188 days incubation, at titers of 2.5 log PFU per gland. For Cs. inornata, NOR virus was first detected at 27 days, and again at 124, 166 and 217 days, when virus titers were 3.3 log PFU per salivary gland. For A. communis, NOR virus was detected after 91 days incubation at 4°C following injection, and after 188 days following injection or 183 days incubation after oral feeding, when virus titers in salivary glands in both batches of mosquitoes attained 2.5 log PFU.

(D. M. MCLEAN).

REPORT FROM THE DEPARTMENT OF TROPICAL MEDICINE AND MEDICAL MICROBIOLOGY,
JOHN A. BURNS SCHOOL OF MEDICINE, UNIVERSITY OF HAWAII, HONOLULU, HAWAII

Enhancement of the infectivity of Murray Valley Encephalitis virus by specific antisera produced in domestic fowls: Proposed mechanism.

Studies on the mechanism of antibody mediated enhancement of plaque formation of Murray Valley Encephalitis virus (MVE) on chick embryo fibroblast (CEF) monolayers as described by Hawkes (1964) have been undertaken.

MVE was mixed with serial dilutions of chicken anti-MVE and normal chicken serum and assayed for plaque formation on chick embryo fibroblast (CEF) monolayers. At dilutions of anti-MVE above the neutralization end point, the number of plaques observed was two to four fold greater than those in normal serum and diluent controls. When the same mixtures were assayed on stable monkey kidney cells (LLC-MK_2), no plaque enhancement was observed. Similar experiments using MVE antiserum prepared in rabbits and assayed on CEF and LLC-MK_2 monolayers demonstrated neutralization but not enhancement.

Preliminary studies indicated that most enhancing activity resided in the IgG fraction and that intact Ig molecules were required for the induction of enhancement. Cleavage of the Fc portion from intact IgG molecules did not inhibit neutralization of virus but abrogated plaque enhancement on CEF monolayers, as did pretreatment of the monolayers with aggregated normal chicken Ig. Aggregated human Ig failed to affect plaque enhancement on CEF monolayers indicating that both enhancement and its inhibition required a degree of taxonomic specificity. This requirement for taxonomic homology at the class level between sources of antisera and cultured cells further suggested the involvement of an interaction between the Fc portion of immunoglobulin and Fc receptors on the cell membrane.

Normal chicken peripheral blood leukocyte cultures (PBL) were unable to support MVE infection. When chicken-anti-MVE serum at dilutions above the neutralization end-point was added to the virus-PBL mixture, greatly enhanced production of MVE was observed. Rabbit-anti-MVE serum failed to augment production of virus in chicken-PBL cultures. But in human-PBL cultures, rabbit-anti-MVE serum at non-neutralizing dilutions did cross enhance dengue virus production while chicken-anti-MVE serum did not. Thus, in both the CEF and PBL culture systems, taxonomic homology at the class level was required for enhanced viral reproduction.

A sub-population of cells comprising CEF monolayers was found to possess Fc receptors as shown by their ability to form EA rosettes. The formation of EA rosettes was also shown to require taxonomic class homology between the Fc donor and the source of receptor-bearing cells. These observations, together with the results of the above enhancement studies, strongly suggest that the Fc bearing sub-population of CEF cells is responsible for antibody mediated enhancement of MVE plaque formation. These cells are likely to be mononuclear phagocytes which probably originating from bone marrow, blood or spleen, of the 11-day chick embryos which were minced to produce the CEF monolayers.

The above findings are consistent with the model of antibody mediated enhanced infection of mononuclear phagocytes by dengue virus described in human and monkeys by Halstead (1977). The phenomenon, obviously of considerable adaptive advantage to the virus, may be a common mechanism in the pathogenesis of many viral diseases whose target cells possess phagocytic capabilities.

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REPORT FROM THE DEPARTMENT OF VIROLOGY AND RICKETTSIOLOGY
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Japanese Encephalitis in 1978 in Japan.

The summer of 1978 was unusually warm through July and August in the mainland of the Japanese Islands. Consequently, high emergence of Culex tritaeniorhynchus, the main vector of Japanese encephalitis in Japan, was reported in Nagasaki, Osaka and Miyagi Prefectures where the emergence of mosquitoes had been monitored every year.

Eighty-eight human cases including 21 deaths were confirmed serologically as JE. Eight deaths were judged to be in presumptive cases on the basis of typical clinical findings. Seventy-four cases including 20 deaths were from southern Kyushu and Shikoku Islands. This number composed 84 per cent of the total cases. The age distribution of the cases was as follows:

Ages	- 80	- 70	- 60	- 50	- 40	- 30	- 20	- 10	- 0
Cases	2	17	22	14	15	2	7	1	8
Deaths	1	9	7	2	1	0	1	0	0

The mortality rate was calculated to be 23.9 per cent.

An enzootic of JE in pigs started in Okinawa in the end of June and spread gradually to the north, involving most of the country except Hokkaido by the middle of September. The enzootic was noted to be especially heavy in the western half of the main Japanese Island. It was shown that more than 80 per cent of pigs had been infected by the end of the enzootic there.

Most of the human cases had no history of vaccination.

(Akira Oya)

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

1. Virus isolation from field caught mosquitoes using virus-sensitive clone C6/36 of Aedes albopictus cells (Singh)

In collaboration with Drs. K. Buei, N. Ueba, and M. Yoshida of the Osaka Prefectural Institute of Public Health, attempts were made to establish the method of virus isolation from field caught mosquitoes using virus-sensitive clone C6/36 of A. albopictus cells. During the summer of 1978 (July 10 till September 11), mosquito specimens were collected at 5 locations of either pigsty (B, D, and I) or cowshed (A, and C). Altogether 32,812 female Culex tritaeniorhynchus mosquitoes in 349 pools were processed. Each pool (not exceeding 100 mosquitoes) was homogenized in 2 ml of 0.2 % bovine plasma albumin in PBS and was centrifuged (10,000 rpm, 30 min). The supernatant was inoculated intracerebrally into a litter of suckling mice (0.02 ml/brain). The remainder of the supernatant was diluted 1:2 and was passed through Millipore HA filter before inoculation into a tube culture of C6/36 cells (0.1 ml/tube), which were then maintained under fluid medium (2 % fetal calf serum in Eagle's medium supplemented with 0.2 mM each of nonessential amino acids) at 28°C. The specimens diluted 1:10 were inoculated to C6/36 cells in Petri dhshes (0.2 ml/60 mm dish) to form plaques under agar overlay.

Brains of morbibound mice were examined by fluorescent antibody staining (FA) to detect antigens of Japanese encephalitis virus (JEV). Infected culture fluids were harvested from tube cultures 7 days after inoculation of the test specimens and were tested for the presence of heamgglutinins with goose red blood cells and virus antigens by complement-fixation test. The fluids were also inoculated to C6/36 cells in 8-chamber slides, which were harvested a few days later to examine virus antigen by peroxidase-anti-peroxidase staining (PAP).

Specimens which were positive by FA, CF or PAP were considered as candidates of JEV isolates and were tested by HI test with standard JEV antiserum. Identification of the isolates were performed by focus reduction neutralization on BHK21 cells using PAP staining technique. JEV was isolated from July 24 until August 21.As summarized in Table 1, C6/36 cells have almost the same or possibly better efficiency of JEV isolation compared with suckling mouse inoculation. Two strains of Getah virus were isolated by C6/36 cells but not by mouse inoculation. Also many viruses were detected by plaque formation on C6/36 cells, however, these viruses have not been identified yet.

Table 1. Summary of JEV Isolation

A. albo. cells	SMB	No. of pools
+	+	24
+	-	9
+	#	5
+	?	1
-	+	4*

Observation failed due to nonspecific death of mice

? Brains were not examined by FA

* Three of the specimens were frozen after inoculation into mice and filtraion before inoculation to C6/36 cells. Another specimen produced some interfering agent in C6/36 cells which reduced the yield of Nakayama strain of JEV.

2. RNA and protein syntheses in A. albopictus, C6/36,cells infected with chikungunya virus under cystine free medium

Studies were made to examine whether cystine deficiency in the medium affects viral RNA and protein syntheses in chikungunya virus-infected A. albopictus, C6/36, cells. Viral RNA synthesis which had been inhibited under cystine free medium was restored to normal level 12 hours after addition of cystine to the medium accompanied by the production of infective virus. Polypeptide synthesis in cystine free medium was generally inhibited except 74K and 83K polypeptides both in infected and uninfected cells. The pattern of polypeptide synthesis was restored to normal 12 hours after adding cystine to the medium.

(Akira Igarashi)

REPORTS FROM THE DEPARTMENT OF MICROBIOLOGY
AND DEPARTMENT OF MEDICAL ZOOLOGY,
KOBE UNIVERSITY SCHOOL OF MEDICINE, KOBE, JAPAN

Multiplication of Dengue Virus in the Salivary Glands of *Aedes aegypti* Intrathracically Infected with the Virus :

Aedes aegypti mosquitoes were infected with dengue virus by the intrathracic injection. At appropriate time after the infection, the salivary glands were excized, fixed with glutaraldehyde and osmium tetraoxide, embedded in epon and examined electron-microscopically.

The data obtained up to the present time are summarized as follows :

(1) The salivary glands at 7 days after infection showed a few dengue-like particles attached to the surface of some acinar cells. However, no mature virion was observed in the cytoplasm of cells.

(2) The salivary glands obtained at 14 days postinfection contained numerous mature virions which were seen in the vesicles and cysternae of the acinar cells.

(3) The maturation of virus at the early stage proceeded mainly in the cytoplasmic matrix, in association with the endoplasmic reticulum.

(4) The viral aggregates were apparently demarcated by vesicular membrane of the cytoplasm. Then, the viral particles in the vesicles were mixed with secretory substances in cytoplasmic vacuoles and excluded into the duct of the salivary glands.

These morphological characteristics were essentially the same as those seen in mammalian culture cells, such as BHK-21, Vero and IMR. However, the viral aggregates seen in the mosquito acinar cells during the final stages were not noted in the above-mentioned mammalian cells, therefore it seemed so far that the viral aggregates were specific for the multiplication processes in the mosquitoes. (This study was done in collaboration with Dr. D.J. Gubler and Dr. L. Rosen.)

(T. Matsumura and S. Hotta)

(S. Hotta)

REPORT FROM THE VIROLOGY AND ENTOMOLOGY DIVISIONS
U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2
TAIPEI, TAIWAN*

1. Further isolation of JE virus (JEV) and isolation of Getah Virus (GET) from the Republic of the Philippines.

A. JEV

We have previously reported the isolation of JEV from Ilocos Sur Province, Luzon, of the Republic of the Philippines. We have more recently made further recoveries of JEV from mosquitoes obtained in San Jose, Nueva Ecija Province, Luzon, R.P. The isolates (Table 1) were obtained either in Vero or BHK_{21} cells using delayed plaquing technique and/or in Ae. albopictus cells using fluorescent antibody staining to indicate the presence of virus. Isolates were identified using a microneutralization test employing a 1.5-2.5 $\log_{10}$ virus challenge to diluted reference HMAF'S. Neutralization test Reactions were much the same as those of the two isolates of JEV reported in the last Information Exchange.

These additional isolations of JEV serve to further confirm the presence of JEV on the Island of Luzon, R.P. There are apparently no reports of encephalitis due to JEV in the R.P. and it is unknown whether this is due to low virulence of Philippine strains of JEV, possible cross-protection or disease modification by other group B arboviruses, or by failure to diagnose and report cases.

B. GETAH

A virus which we have identified as Getah was isolated from mosquitoes also collected at San Jose (Table 1). A pool of 80 C. vishnui caused plaque formation in both Vero and BHK_{21} cells. This isolate was passed and a HMAF prepared. Cross-microneutralization tests indicate that the isolate (Ph Ar-814) most closely resembled GET virus (Table 2) although it is also closely related to Sagayama virus. This is apparently the first reported isolation of GET virus in the R.P. although it has been found previously in Malaysia, Cambodia, Japan, and Australia.

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2. Neutralizing JEV antibody in horses at Subic Bay Naval Base (SBNB), R.P.

Sera of 36 horses at the riding stables at SBNB were tested for HI and neutralizing antibody using standard HI techniques and a microneutralization test using between 1.5-2.5 $\log_{10}$ of the Nakayama strain of JEV.

The results of the tests are summarized in table 3. The presence of neutralizing JEV antibody in these horses which have been, for the most part, imported (16 from the U.S., 8 from Australia) or born on the SBNB (10) indicates that JEV to present and is being transmitted to the horses at this location. All five yearling and two year olds tested did not have neutralizing antibody. There have been no epizootics of encephalitis among the horses at the riding stable at SBNB within recent memory even though JEV has been reported to cause encephalitis in horses in other areas of Southeast Asia.

T. G. Ksiazek, NAMRU-2, R. Bredlow, SBNB.

3. Use of a microneutralization test for serologic testing of Southeast Asian Group B arbovirus.

We have recently been utilizing a microneutralization test for testing sera positive by HI test to attempt to determine which group B arbovirus was responsible for the presence of HI antibody. The test we are now employing uses M-199 medium with Hank's Salts buffered with 0.01 M Tricine and 0.01 M NaHCO3. This medium allows the microplates to be incubated without sealing or using a CO2 incubator. For the Gp B viruses we have found that the PS line of porcine kidney cells have consistantly given higher virus titers than Vero, LLC-MK_2, or BHK_{21} cells and that the CPE produced is much more readable.

Neutralizing tests are performed by diluting heat inactivated sera using 0.025 ml microdiluters starting at a serum dilution of 1:5. Care must be taken not to scratch the plates with the microdiluters. A 0.025 ml drop containing 1.5 to 2.5 $\log_{10}$ of test virus in Hank's balanced salt solution with 0.5% gelatin was added to the diluted sera to be tested and, after mixing, incubated at 35°C for 1 hour. Two 0.05 ml drops of PS cells (90,000 cells/ml) were then added to each well. The plates are then incubated for a predetermined period which corresponds for each virus to the time necessary for maximal CPE development in virus titrations. A back titration of virus performed with each test indicates the actual test dose of each virus.

The test offers a great deal of specificity in distinguishing Gp B arboviruses when four injection HMAFs are used (Table 4). The test may offer diminished sensitivity when compared to that of the PRNT (2 to 4 fold differences in titer). However, the ease of performance of the test and ability to test large numbers of sera using many viruses, in our opinion, make the test a suitable substitute for the PRNT. The ease of obtaining neutralizing antibody titers to many viruses instead of qualitative data as is often done with the PRNT has enabled us to distinguish the group B virus responsible for infection in many instances. However, in areas of multiple Gp B infections the situation is often still too confusing to enable clear interpretation with some sera tested.

T. G. Ksiazek, L. Liu, F. M. Fu.

Table 1. Mosquitoes collected near San Jose, Nueva Ecija Province, Luzon, Republic of the Philippines during August 1977.

Species	mosquitoes (%)	No. of pools	No. of positive pools
Culex vishnui	50,652 (63.8)	525	1 *
Cx. tritaeniorhynchus	11,396 (14.4)	139	1 **
Cx. fuscocephala	4,183 (5.3)	57	0
Cx. annulirostris	2,801 (3.5)	33	0
Cx. bitaeniorhynchus	1,744 (2.2)	29	1 **
Cx. whitmorei	1,734 (2.2)	28	0
Cx. gelidus	820 (1.0)	14	0
Cx. fuscanus	117 (0.2)	4	0
Cx. fatigans	36 (0.0)	1	0
Anopheles annularis	2,491 (3.2)	32	1 **
An. peditaeniatus	1,936 (2.5)	27	0
An. indefinitis	300 (0.4)	3	0
An. tessellatus	220 (0.3)	4	0
Aedes vexans	276 (0.4)	7	0
Ae. lineatopennis	164 (0.2)	3	0
Mansonia uniformis	307 (0.4)	7	0
Total	79,177 (100.0)	913	4

* GET virus isolate, pool of 80 C. vishnui

** JEV isolates, C. tritaeniorhynchus, pool of 50; C. bitaeniorhynchus pool of 28; An. annularis pool of 92.

Table 2. Results of a microneutralization test of isolate Ph Ar 814 (pool of Culex vishnui).

HMAF	Virus			
	814	SAG	RR	GET
814	80	80	10	320
SAG	640	2560	40	1280
RR	320	1280	≥5120	1280
GET	320	1280	40	1280

* Hyperimmune mouse ascitic fluid (four dose).

The virus was also tested against the following HMAF's and found to have titers < 1:10: BEB, WHA, EEE, WEE, VEE, SIN, WN, TMU, LGT, MVE, ZIKA, DEN 1-4, Y.F., SLE, BUN, SEP, KUN, BAT, BAK, ING, UMB, normal MAF.

Table 3. Distribution of JEV HI and Nt Antibody titers among 36 equines at the riding stables, U.S. Naval Base, Subic Bay, Republic of the Philippines.

Titer	Micro-Nt (%)	HI (%)
Neg	8 (22.2)	14 (38.9)
1:5	3 (8.3)	ND
1:10	2 (5.6)	0 (0)
1:20	5 (13.9)	4 (11.1)
1:40	8 (22.2)	10 (27.9)
1:80	5 (13.9)	7 (19.4)
≥1:160	5 (13.9)	1 (2.8)
Total	36 (100)	36 (100.0)

Table 4. Results of Cross-Neutralization tests employing a micro-neutralization procedure.***

HMAF	VIRUS									
	JE	MVE	SLE	WN	KUN	DEN-2	ZIKA	SEP	LT	TMU
JE	160**	20	-	-	-	-	-	-	-	-
MVE	- *	320	-	-	-	20	-	-	-	-
SLE	-	-	160	-	-	-	-	-	-	-
WN	-	-	-	640	640	-	-	-	-	-
KUN	-	-	-	160	320	-	-	-	-	-
DEN-2	-	-	-	-	-	240	-	-	-	-
ZIKA	-	-	-	-	-	-	5120	-	-	-
SEP	-	-	-	-	-	-	-	640	-	-
LGT	-	-	-	-	-	-	-	-	320	-
TMU	-	-	-	-	-	-	-	-	-	320
DEN-1	-	-	-	-	-	60	-	-	-	-
DEN-3	-	-	-	-	-	20	-	-	-	-
DEN-4	-	-	-	-	-	20	-	-	-	-
YF	-	-	-	-	-	-	-	-	-	-

* = $<$1:20

** Reciprocal of highest dilution neutralizing 1.5-2.5 $\log_{10}$ of test virus.

*** In addition HMAFs to the following viruses were tested against the viruses listed and found to have neutralizing titers of $<$ 1:20; CHIK, RR, GET, BEB, WHA, EEE, WEE, VEE, SIN, SAG, ING, BUN, and normal MAF.

REPORT FROM THE VIRUS LABORATORY
INSTITUTE OF PUBLIC HEALTH
UNIVERSITY OF THE PHILIPPINES, MANILA

RAPID AND EARLY DIAGNOSIS OF DENGUE HAEMORRHAGIC FEVER BY THIN-LAYER IMMUNOASSAY

A study on the applicability of thin-layer immunoassay (TIA) for the rapid and early diagnosis of dengue haemorrhagic fever (DHF) is in progress.

In response to the need for a rapid and sensitive test for the early detection of DHF cases, antigen detection rather than antibody was pursued. The method is a modified version of that of Elwing, Nilsson and Ouchterlony (1). It consists of adsorbing antidengue antibodies on polyacrylic plastic sheets of any desired size. On this antibody lawn, acute serum of patient is deposited by a micropipeter, and the reaction is allowed to take place in a humidified chamber at 37°C. for one hour. Screening is done on undiluted and 1:10 dilution of patient serum. The reaction is stopped by exposing the plate over steam at 60°C. for 60 seconds and then washed with distilled water. Slow air current is used to dry the plates. Reading is based on the formation of condensed water vaper when plates are held over steam at 60°C. Positive reaction is seen as clear spots produced as a result of the formation of large confluent condensed water vaper drops, while negative reaction is a hazy spot indistinguishable from the background due to small dreps of condensed water vaper.

PRELIMINARY RESULTS:

As was presented in the WHO Third Technical Advisory Committee Meeting on DHF held in Manila on 6-8 December, 1978, TIA looks like it is going to be a promising test for the rapid and early diagnosis of DHF. It is inexpensive, easy to perform in the laboratory and we imagine even in the field, and it is as sensitive as HI test. Its specificity will depend on the availability of monospecific antisera for the four dengue serotypes which according to suggestions given in the meeting can be produced by hybridomas.

Initially 34 acute sera were tested from clinically diagnosed DHF cases whose severity of haemorrhage were graded according to WHO criteria (Table I-A). Of these, 16 had unpaired or single serum and 18 had paired sera. Twenty two other cases were included (Table I-B) such as 12 bacterial and 10 other viral infections. Age and sex of the 2 groups were matched as closely as possible.

Table II-A shows that 76.5% (26/34) of clinically diagnosed DHF cases were positive for dengue antigen on antibody lawn using 2 units of HI antidengue 2 antibodies. The TIA results of the other group are shown in Table II-B. Bacterial infections were totally negative, while 4 out of 10 other viral infections gave positive results. These 4 cases were in most probability true dengue infections that were misdiagnosed.

Correlation of TIA and HI results are presented in Table III. There is good correlation between the two tests, but, it seems that when sera have high HI titers (greater than 1:40), TIA is likely to miss detection of dengue antigen. Such a drawback is understandable if one thinks of immune complex formation. On the other hand, drawing two blood samples for serology is often impossible, thus TIA offers an advantage in that diagnosis can already be made from the single blood sample as in 68.75% (11/16) of the cases with unpaired serum samples. Furthermore, diagnosis can be made as early as possible which is of great help to the attending physician so that patients who might go into shock can be managed accordingly.

Further studies such as effect of antibody concentration used in the preparation of antibody lawn on the sensitivity of the test, and shelf life of the antibody lawn are being undertaken.

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Acknowledgement:

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Table 1 - A

Study Group For TIA Testing Composed of Clinically Diagnosed DHF Cases

Hemorrhagic Severity	Cases With Unpaired Blood (Acute Phase Only)	Cases With Paired Blood
Grade I	8	4
Grade II	8	9
Grade III	0	5
Grade IV	0	0
T-O-T-A-L	16	18

Table 1 - B

Control Group For TIA Testing

Clinically Diagnosed Viral Infections		
	1. Systemic Viral Infection	3
	2. Rubeola	2
	3. Acute Upper Respiratory Disease	4
	4. Enteroviral Infection	1
Bacterial Infection		
	1. Salmonella Infection	7
	2. Streptococcal Rheumatic Infection	5
T-O-T-A-L		22

Table II - A

Detection of Dengue Antigen By TIA on Clinically Diagnosed DHF Cases

Hemorrhagic Severity	Acute Phase Sera With ≤ 1:40 HI Titer		Acute Phase Sera With >1:40 HI Titer	
	TIA neg.	TIA Pos.	TIA Neg.	TIA Pos.
Grade I	3	7	0	2
Grade II	4	12	0	1
Grade III	0	4	1	0
Grade IV	0	0	0	0
T-O-T-A-L	7	23	1	3

Total Negatives	8/34	=	23.5
Total Positives	26/34	=	76.5

Table II - B

TIA Results of the Control Group

Viral Infections	No. Examined	TIA Positive
Systemic Viral Infection	3	2
Rubeola	2	1
Acute Upper Respiratory Infection	4	1
Enteroviral Infection	1	0
Bacterial Infections		
Salmonella	7	0
Streptococcal Rheumatic Fever	5	0

Table III

Correlation of TIA and HI Test Results

	HI Test	TIA Positives
Paired Samples of DHF Cases		
Positive Dengue Infection by HI Test		
$\leq$ 1:40 Titer of Acute Sample	10	10
$>$1:40 Titer of Acute Sample	4	3
Presumptive Dengue Infection by HI ($<$20 Acute, 1:20 Conv.)	4	2
Unpaired Samples of Clinically Diagnosed of DHF Cases		
$<$1:20	8	5
1:20	2	2
1:40	6	4

REPORT FROM CSIRO DIVISION OF ANIMAL HEALTH,
PRIVATE BAG NO. 1, P.O., PARKVILLE, VICTORIA, AUSTRALIA.
IN COLLABORATION WITH ANIMAL VIRUS RESEARCH INSTITUTE,
PIRBRIGHT, WOKING, SURREY, ENGLAND.

Since the identification of CSIRO-19 (isolated from a mixed pool of Culicoides caught at Beatrice Hill, Northern Territory) as related to the bluetongue group of viruses, by the grouping complement-fixation (CF) test, we have been involved in extensive serological studies with this virus. This has involved looking at different growth systems, isolation systems, production of CF antigen and serological comparisons with the bluetongue virus (BTV) serotypes and with other Australian orbiviruses.

It has been found that a more satisfactory CF antigen for detecting BTV infections can be produced from infected cell cultures than from infected mouse brain. CSIRO-19 virus was found to produce highest yields in African green monkey kidney (VERO) cells with good yields in baby hamster kidney (BHK-21) cells and primary and secondary ovine kidney cells. The cold fixation method (4°C for 18 hours) was found to be much more satisfactory than the warm fixation method (37°C for 90 minutes). Infectious virus in the CF antigen was inactivated with 0.2% β-propiolactone. Large scale production of the inactivated CF antigen was undertaken in order to produce stocks for State diagnostic laboratories. Sufficient bluetongue virus (CSIRO-19) CF antigen to perform 2,500,000 tests was distributed in 1978.

Intravenous inoculation of 11 day-old chick embryos with CSIRO-19 was studied, as this has been found to be more sensitive than cell culture systems for isolation and assay of other BTV types. CSIRO-19 virus has been passaged through eggs by the intravenous route. High titres of virus were not found in the chick embryos, even after as many as six passages. The chick embryo was also found to be much less (10,000 - fold) sensitive for the detection of CSIRO-19 than cell culture systems. Thus, CSIRO-19 is unlike other BTV types in that it does not grow well in the chick embryo and that cell culture systems are more sensitive for the assay of virus infectivity.

Comparison of CSIRO-19 (designated as a new type, BTV-20, by the World Reference Laboratory, Onderstepoort, South Africa) with BTV types 1 to 17, Ibaraki and epizootic haemorrhagic disease of deer (New Jersey) viruses were undertaken using a microtitre neutralization test. CSIRO-19 could not be distinguished from BTV-4 and antisera against CSIRO-19 neutralized BTV-17. Extensive comparisons were done using plaque-inhibition and plaque-reduction tests, both of which were more sensitive than the microtitre neutralization test. The plaque-inhibition test proved most useful in these comparisons and confirmed the two-way serological relationship between CSIRO-19 and BTV-4 and also that BTV-17 was neutralized by CSIRO-19 antiserum, but CSIRO-19 virus was not neutralized by BTV-17 antiserum. Other more recently isolated BTV-4 viruses from Cyprus also exhibited similar one-way relationships with BTV-17. Plaque-reduction assays, using variable virus and serum levels demonstrated that although related, CSIRO-19 could be distinguished from BTV-4 by its neutralization curve.

A number of Australian orbiviruses, not directly related to bluetongue virus, appear to produce serological cross-reactions which may be of importance when interpreting serological tests. Bovine antisera and hyperimmune mouse ascites tumour fluids were prepared against a number of Australia orbiviruses.

Of eighteen hyperimmune ascites fluids prepared in mice against Eubenangee virus (strain BHO 754), that produced in one mouse showed a strong agar gel precipitin reaction (group test) against CSIRO-19 antigen, with several others showing weak reactions. Of three cattle inoculated with D'Aguilar virus (strain 4204), one animal showed a serum neutralizing response to CSIRO-19 detected by plaque-inhibition tests. Studies with cattle sera produced against Eubenangee virus suggested that there was a low level one-way neutralization relationship with BTV-1. Studies are underway to more thoroughly investigate the relationships between the Australian orbiviruses and the members of the bluetongue virus complex.

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REPORT FROM THE JAKARTA DETACHMENT
OF THE U.S. NAVAL MEDICAL RESEARCH UNIT NO. 2,
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1. Serologic evidence of arboviral infections causing fever in Central Java.

The University of Gajah Mada, Yogyakarta, Indonesia with support from the Rockefeller Foundation completed a study of febrile patients hospitalized in Klaten, Central Java. Criterion for inclusion in the study was an axillary temperature of 38° Celsius on examination and a history of 3 or more days of fever. The primary objective of the study was to determine the relative importance of leptospirosis as a cause of fever in the population served by this hospital.

Serologic studies were completed by NAMRU-2 including the leptospiral passive hemagglutination test using methods described by Sulzer, et al, (1975). A total of 5 patients (7%) of 68 tested showed a diagnostic ($\geq$ 4-fold rise in titer) or presumptive (standing titer $\geq$ 1:640) evidence that infection with Leptospira spp. was the cause of their fever.

Acute and convalescent sera from 121 patients were tested by hemagglutination inhibition (HI) for evidence of infection with alphaviruses and flaviviruses. Each pair of sera was tested with hemagglutinating antigens (HA) prepared from Japanese encephalitis (JE), Nakayama; dengue type 2 (DEN-2), New Guinea C; Chikungunya (CHIK), S-27 and Ross River (RR), T-48, viruses were obtained from the National Institute of Allergic and Infectious Diseases (NIAID).

Of the 121 pairs of sera, 56 (46%) showed positive ($\geq$ 4-fold rise) or presumptive ($\geq$ 1:640) evidence of infection with one or more HA used in the test. A total of 44/121 (36%) of the patients had evidence of a group B infection. In addition, 21/121 (17%) showed evidence of infection with a group A arbovirus.

Although many sera had antibody to more than one HA, some had antibody to only one HA. Of the 26 sera which showed positive or presumptive evidence of infection with only one virus, 13/121 (11%) were to JE, 5/121 (4%) to DEN-2, 6/121 (4%) to CHIK and 2/121 (2%) to RR.

Microneutralization (Nt) tests were performed on 42 pairs of sera which had shown presumptive or positive HI test results (Tables 1 and 2). The following virus strains were used in the tests: JE, Nakayama; DEN-2, New Guinea C; Zika, MR 766; Tembusu (TMU), MM 1775; Murray Valley encephalitis (MVE), Original; CHIK, S-27; RR, T-48; Getah (GET), MM 2021, and Sindbis (SIN), AR 339. A total of 28 patients had diagnostic increases of Nt antibody from their acute to convalescent phase sera: seven had DEN-2, 10 had Zika, 4 had CHIK, and 7 showed 4-fold rises to both DEN-2 and Zika.

Suhandiman, Suwardji, and Rohde, J.

2. Prevalence of neutralizing antibodies in sera collected from domestic animals resident in Lombok, Indonesia.

Sera collected from 235 domestic animals (16 equines, 41 bovines, 13 carabao, 35 goats, 77 chickens and 53 ducks) were assayed for antibodies to JE, ZIKA, CHIK and RR by HI test. Eight of 16 equines (50%), 9 of 41 bovines (22%), 1 of 13 carabao (8%) and 10 of 35 goats (29%) had antibody to JE or ZIKA. None of the sera collected from domestic mammals had antibody to CHIK or RR. Sera from 24 of the 28 animals which were positive for group B antibody were tested by microneutralization using JE, ZIKA, MVE, TMU, LGT, KUN, SEP and DEN-2 viruses.

Five of the original 6 sera from horses (2 were contaminated) had significant Nt antibody titers ($\geqslant$ 1:40) to group B arboviruses; 3 to SEP, and 1 each to JE and KUN. Six of 8 sera from cattle (1 serum was contaminated) and the single serum from a carabao had antibody to SEP. Eight of 9 goat sera (1 was contaminated) had antibody to group B arboviruses; 4 of 9 to SEP, 3 of 9 to MVE and 1 to KUN. Prevalence of antibody by species and group B arboviruses are shown in table 3.

Sera from 41 domestic animals were tested by Nt test using Bunyamwera, Batai and Bakau viruses. Results of these tests are shown in table 4. Batai virus infection is common among cattle (100%) and also occurred in carabao (25%), goats (17%) and chickens (6%).

Gindo Simandjuntak, Sustriayu Nalim, Omik Koswara, J. G. Olson, T. G. Ksiazek.

3. Prevalence of neutralizing antibodies in the sera of wild and domestic animals in Sumatra, Indonesia.

Sera were collected animals resident in Way Abung III in Lampung Province, Baturaja in South Sumatra Province and Singkut in Jambi Province. Each area is a government transmigration site where families from heavily populated Java are relocated. Sera were first tested by HI for antibodies to JE, CHIK, RR and GET. Those sera which had measurable HI titers ($\geqslant$ 1:10) were then tested by microneutralization for antibodies to alphaviruses and flaviviruses. In addition sera were tested for neutralizing antibodies to three Bunyamwera group viruses.

Sera collected from 67 cattle were tested for antibodies to group B arboviruses in a JE HI test. Twenty-four of the sera had measurable HI titers and were then tested by microneutralization for antibodies to JE, DEN-2, ZIKA, MVE, KUN, SEP, LGT, and TMU. Table 5 shows the results of those tests. Nearly all the sera (88%) which had HI antibody to JE had Nt titers to one or more flaviviruses tested. Only 4 sera had no significant titer to any of the agents. A total of 14 of the 67 (21%) had JE antibody, 11 (16%) had SEP antibody and 1 had antibody to TMU.

Some sera had antibody to only a single virus (14) and others had antibody to more than one virus (6). Eight sera had antibody to JE only, 6 to SEP only, 5 to JE and SEP and one to JE and TMU.

Two cattle of the 67 tested had HI titers against one or more group A arboviruses (CHIK, RR, and GET). Only one of these had a significant Nt antibody titer to both RR and GET when tested using CHIK, RR, GET and SIN.

A total of 174 sera from domestic and 98 wild animals were assayed to Nt antibodies to BUN, BAT and BAK. Evidence of infection with Bunyamwera group viruses was found in 40 of 67 (60%) of cattle, 10/39 (26%) of goats, 2/15 (13%) of carabao and 2/53 (4%) of chickens tested. None of the 26 wild avians or 62 rodents had antibody to any of the 3 Bunyamwera group viruses used in the test. Nearly all infections were with BAT, one chicken had antibody only to BAK and 6 cattle had significant titers ($\geqslant$1:40) to BUN but each of these sera also had higher titers of antibody to BAT. It is likely that animals infected with BAT or a closely related virus develop antibody which cross reacted with BUN in our neutralization tests.

Sustriayu Nalim, Iwan Budiarso, J. G. Olson, T. G. Ksiazek.

Table 1. Hemagglutination inhibiting and neutralizing antibodies to flaviviruses in acute and convalescent sera of patients with febrile illnesses.

SERUM NO.	HI TITER* JE	HI TITER* DEN-2	Nt TITER* JE	Nt TITER* DEN-2	Nt TITER* ZIKA	Nt TITER* TMU	Nt TITER* MVE
5757 A	40	160	-	10	10	-	-
C	40	640	-	10	80	-	-
5769 A	10	160	-	-	80	-	10
C	40	160	10	10	≥160	-	20
5171 A	10	40	20	20	≥160	-	10
C	40	80	-	20	≥160	-	10
5783 A	-	-	-	-	20	-	-
C	320	320	-	80	≥160	-	20
5793 A	-	-	-	-	10	ND	10
C	20	40	-	10	40	ND	-
5795 A	320	2560	-	40	80	ND	10
C	640	640	-	40	80	ND	10
5803 A	10	20	-	-	10	ND	-
C	40	320	-	10	40	ND	-
5807 A	40	80	-	40	20	ND	10
C	160	320	-	20	≥160	ND	10
5809 A	40	20	-	10	10	ND	-
C	160	320	-	40	40	ND	10
5821 A	20	40	-	40	ND	ND	-
C	640	1280	-	≥160	80	ND	-

Table 1 (cont'd)

SERUM NO.		HI TITER* JE	HI TITER* DEN-2	Nt TITER* JE	Nt TITER* DEN-2	Nt TITER* ZIKA	Nt TITER* TMU	Nt TITER* MVE
5823	A	40	80	-	20	≥160	-	-
	C	2560	≥5120	-	≥160	≥160	-	10
13164	A	640	160	10	40	10	10	20
	C	160	80	10	80	≥160	20	20
13168	A	80	80	-	40	≥160	20	-
	C	>5120	2560	10	≥160	≥160	40	10
13180	A	80	80	-	40	80	-	10
	C	320	160	-	20	80	-	10
13200	A	20	20	-	-	40	-	10
	C	640	640	-	80	≥160	10	-
13204	A	40	20	20	-	-	10	-
	C	640	320	10	20	40	20	10
13208	A	20	20	-	20	10	-	10
	C	5120	2560	-	≥160	40	-	20
13214	A	10	10	20	-	-	10	-
	C	160	40	10	20	40	20	10
13216	A	20	20	-	20	20	10	-
	C	5120	2560	-	≥160	≥160	20	-
13218	A	40	40	10	-	≥160	10	10
	C	1280	1280	10	≥160	≥160	20	20
13228	A	80	20	-	10	10	-	-
	C	40	80	-	20	10	-	-
13230	A	320	320	-	80	≥160	10	-
	C	2560	2560	10	≥160	≥160	20	20

Table 1 (cont'd)

SERUM NO.		HI TITER* JE	HI TITER* DEN-2	Nt TITER* JE	Nt TITER* DEN-2	Nt TITER* ZIKA	Nt TITER* TMU	Nt TITER* MVE
13236	A	80	40	-	40	⩾160	-	-
	C	160	160	-	80	40	10	10
13254	A	160	80	-	-	10	10	-
	C	1280	640	-	⩾160	10	-	10
13256	A	160	40	-	20	20	10	10
	C	320	160	10	40	⩾160	-	-
13258	A	10	10	-	-	-	-	-
	C	320	160	10	40	80	10	10
13268	A	320	80	-	-	20	-	40
	C	2560	640	40	⩾160	⩾160	20	10
13270	A	40	40	-	40	10	10	10
	C	320	160	-	40	⩾160	10	-
13272	A	-	-	-	-	-	-	-
	C	160	160	-	20	20	-	-
13278	A	80	80	10	80	10	-	-
	C	320	320	40	80	⩾160	40	20
13282	A	160	80	-	20	⩾160	-	-
	C	320	320	-	⩾160	-	10	10
13284	A	80	40	-	20	40	-	-
	C	320	160	-	⩾160	10	-	-
13286	A	20	20	-	20	20	-	10
	C	80	80	-	20	80	10	10

Table 2. Hemagglutination inhibiting and neutralizing antibodies to alphaviruses in acute and convalescent sera of patients with febrile illnesses.

SERUM NO.		HI TITER CHIK	HI TITER RR	Nt TITER CHIK	Nt TITER RR	Nt TITER GET	Nt TITER SIN
13164	A	-	-	-	-	-	-
	C	80	20	20	-	10	-
13198	A	-	-	-	10	-	-
	C	20	-	80	-	10	-
13200	A	-	-	-	-	-	-
	C	20	-	40	-	-	-
13214	A	-	-	-	-	-	-
	C	40	20	-	-	-	-
13222	A	20	-	20	20	10	-
	C	40	40	-	20	-	-
13226	A	-	-	-	-	-	-
	C	40	-	$\geq$160	-	-	-
13236	A	-	-	-	-	-	-
	C	40	-	$\geq$160	-	10	-
13268	A	-	10	-	10	10	-
	C	40	80	-	-	-	-
13272	A	-	-	-	10	10	-
	C	40	40	-	-	-	-

Table 3. Prevalence of neutralizing antibodies to flaviviruses in domestic animals resident in Lombok.

	No. positive*/No. tested** (%)			
	Horses	Cattle	Carabao	Goats
SEP	3/15 (20)	6/40 (15)	1/13 (8)	4/34 (12)
MVE	0/15	0/40	0/13	3/34 (9)
KUN	1/15 (7)	0/40	0/13	1/34 (3)
JE	1/15 (7)	0/40	0/13	0/34
TOTALS	5/15 (33)	6/40 (15)	1/13 (8)	8/34 (24)

* Sera with HI titer and Nt titer $\geq$1:40 were considered positive.

** Sera tested by HI less those which were positive by HI which were not tested by Nt.

Table 4. Neutralizing antibodies to Bunyamwera viruses in serum of domestic animals resident in Lombok.

	No. positive*/No. tested (%)		
	Bunyamwera	Batai	Bakau
Horses	0/6	0/6	0/6
Cattle	1/5 (20)	5/5 (100)	0/5
Carabao	0/4	1/4 (25)	0/4
Goats	1/6 (17)	1/6 (17)	0/6
Chickens	0/16	1/16 (6)	0/16
Ducks	0/4	0/4	0/4
All species	2/41 (5)	8/41 (20)	0/41

* Antibody titers $\geq$ 1:40 were considered positive.

Table 5. Neutralizing antibodies to flaviviruses in sera from cattle resident in Sumatra.

Serum No.	HI titer* (JE)	Ni titers ** JE	DEN-2	ZIKA	MVE	KUN	SEP	LGT	TMU
16352	20	80	-	-	-	10	≥160	-	-
16356	40	≥160	-	-	20	-	-	-	-
16359	10	10	-	-	-	-	-	-	-
16363	10	≥160	-	10	-	-	80	-	-
16364	10	-	-	-	-	-	40	-	-
16365	20	-	-	-	-	-	≥160	-	-
16375	20	20	-	-	-	-	-	-	10
16384	20	80	-	-	20	-	80	10	-
16385	40	20	-	10	-	-	≥160	10	-
16386	10	40	-	10	-	10	10	10	10
16387	80	≥160	-	10	20	10	20	20	40
16392	40	20	-	10	-	-	40	-	-
16394	20	40	-	10	10	10	≥160	-	-
16396	40	≥160	-	-	-	-	≥160	-	-
16397	20	40	-	20	10	-	20	-	-
16402	20	10	-	-	-	-	10	10	-
16504	10	20	-	10	-	-	80	10	-
16507	20	80	-	10	-	-	10	10	-
16508	20	20	-	10	-	-	≥160	-	-
16515	20	≥160	-	10	10	-	-	-	-
16540	20	40	-	10	-	-	-	-	-
16544	20	80	-	-	-	-	-	10	-
16545	40	40	-	-	-	-	-	-	-
16549	10	20	-	-	-	-	-	-	-

REPORT FROM THE ARBOVIRUS RESEARCH UNIT
UNIVERSITY OF CALIFORNIA INTERNATIONAL CENTER FOR MEDICAL RESEARCH
HOOPER FOUNDATION, SAN FRANCISCO
AND
THE UNIVERSITY OF MALAYA, KUALA LUMPUR

I. Umbre (?) Virus in Malaysia

In past reports, we have identified several virus strains isolated from sentinel chickens and mosquitoes, principally Culex vishnui, as strains of Umbre virus in the Turlock serogroup. Results of miscellaneous neutralization tests in which these viruses were employed, however, indicated that the Malaysian strains may be significantly different from the prototype Indian strain of Umbre. This was confirmed by the results of plaque-reduction neutralization tests in Vero cells comparing four selected Malaysian strains (P71-301, P74-1329, P70-443, P75-465) with the prototype. The viruses tested were all 5th passage Vero cell stocks; the antisera were prepared in mice by five intraperitoneal inoculations.

Virus	Hyperimmune Mouse Sera				
	Umbre	P71-301	P74-1329	P70-443	P75-465
Umbre	2000*	110	80	30	30
P71-301	65	560	240	120	170
P74-1329	35	320	380	320	380
P70-443	40	160	180	320	280
P75-465	150	1800	2200	3400	4200

*Reciprocal of serum dilution showing 50% plaque reduction in Vero cells.

Whether or not the Malaysian strains should be identified as a new Umbre-related virus or simply be considered geographic variants of Umbre is a subjective decision. It should be noted, however, that the differences demonstrated in this cross-comparison are greater than those used in separating some other viruses registered as new in the International Catalogue of Arboviruses.

II. Assessment of Forest Habitats for Potential Dengue Vectors

Seven different types of forest habitats in Malaysia, assessed to date for potential dengue virus vectors, were all characterized by Aedes albopictus diurnal biting activity at ground level. The numbers of this species attacking humans, however, varied considerably among the different types of forest. They were negligible in man-made oil palm plantation to very numberous in man-made rubber plantation. A young entomologist, who studied this species

in parts of Malaysia a few years ago, aptly referred to rubber plantations as "albopictus factories." Despite the numbers of Ae. albopictus active near ground level, very few specimens were taken in the canopy except in a mangrove swamp forest, which had a relatively low canopy. In deep primary hill forest, Ae. albopictus mosquitoes were found in relatively small numbers. They were more abundant in lowland dipterocarp, disturbed hill dipterocarp, freshwater peat-swamp forest, and mangrove swamp forest.

Ae. pseudoniveus and subniveus were the most widespread of the Ae. (Finlaya) niveus subgroup, which we believe contains the forest canopy vectors of dengue. These two species always appeared together when they were collected and were active almost exclusively in the high canopy. They were the most common canopy Aedes collected in primary hill dipterocarp, lowland dipterocarp, and freshwater peat-swamp forests. They were rare in the rubber plantation site.

In man-disturbed hill dipterocarp forest, Ae. (Finlaya) novoniveus was the most common canopy species of the subgroup. This forest had numerous stands of giant bamboo, which provide the preferred breeding places for this mosquito. In mangrove swamp forest, Ae. (Finlaya) litoreus and niveus leonis were the dominant canopy species of the niveus subgroup, while in oil palm no mosquitoes of this subgroup were detected.

The observed differences in the various forest habitats in regard to the activity of these known and suspect dengue vector populations, would indicate variations in dengue virus cycles that would be habitat dependent. The risk of infection to humans in the forest would be directly related to the type of forest and to human activity there (ground vs. canopy).

III. Dengue Virus Activity in Malaysia

Since the beginning of these studies, we have recovered 297 strains of dengue virus in Malaysia, principally from humans but also from monkeys and three species of mosquitoes. All four types are represented. From 1973 to 1978, however, type 3 appeared to be predominant. In 1978 there was an indication, on the basis of isolations from humans, that type 3 activity was diminishing and being replaced by type 2.

IV. Rural Dengue

The results of a pilot study, in which over 1500 children in 20 rural communities were examined for flavivirus antibody, revealed a direct relationship between environment and flavivirus infection in an area where Aedes aegypti is absent, but Aedes albopictus is widespread. Of 584 households in the study, 36 percent were positive. The positives by village, however, ranged from 0 percent (for one) to 83 percent. Three villages had positive rates significantly higher than any of the others. These villages are characterized by low population density, non-mobile individuals, and the same type of environment, i.e., situated in rubber tree areas adjacent to forest. The villages with the lowest rates, in contrast, are characterized by high population density, relatively mobile individuals, and no close association with rubber or forest.

Of the villages with high flavivirus antibody prevalences, one was selected for intensive study, which was recently initiated. To date, three individuals have been detected in routine home visits who have had apparent dengue virus infections, based on serological results of paired blood samples. Two of the cases were characterized by undifferentiated fevers of short duration (2-3 days), while no illness could be recalled for the third. These early results in our investigation of rural dengue, tend to support our belief that rural dengue infection in the absence of Aedes aegypti mosquitoes is clinically mild.

(Albert Rudnick, John J. S. Burton, and William A. Neill.)

REPORT FROM THE DEPARTMENTS OF VIROLOGY, MEDICAL ENTOMOLOGY AND EPIDEMIOLOGY, U.S. ARMY MEDICAL COMPONENT, ARMED FORCES RESEARCH INSTITUTE OF MEDICAL SCIENCES, BANGKOK, THAILAND

Epidemiological and ecological investigations of dengue viruses were initiated in a low socio-economic community of Bangkok, Thailand, during June, 1978, to determine the relation of Aedes aegypti population and behavioral changes to the seasonal incidence of dengue virus infections in humans. Emphasis is also being directed to the possible effects of temperature and the genetics of Ae. aegypti on the seasonal vector capabilities of this species.

Dengue virus infections in the human population.

Serological data and hospital records indicate that dengue viruses are prevalent in the human population of the study area. The seasonal incidence of dengue virus infections is being determined for members of 100 randomly selected families in the study area. Blood specimens are being obtained during the cool and hot seasons and at the beginning and end of the rainy season for determination of seroconversion rates. Overt dengue virus infections are being determined by bi-weekly visits to the residences of the 100 family units.

Currently, blood specimens have been obtained from members of the family units before and after the rainy season of 1978. Plasma are being assayed by hemagglutination inhibition tests to determine seroconversion rates. To date, confirmed cases of overt dengue virus infections have not been detected among the 100 selected families.

Survey for mosquito larval habitats

The availability of larval habitats and their utilization by Ae. aegypti, are being surveyed seasonally for each of the 100 residences. All containers are being searched thoroughly for larvae and pupae of aegypti.

Family residences were surveyed inside and outside during the hot season, 3-18 April, and the early rainy season, 30 May-15 June. Natural habitats were not found during these surveys, as vegetation is practically non-existent in the study area. The most common containers with water were clay ceramic water jars, ant traps, flower vases, tin cans, plastic buckets, pans and cement water basins. A seasonal comparison of the containers with water and those with aegypti is presented in Table 1. There was a considerable increase

in the number of aegypti positive containers, both inside and outside houses, between the dry season and the early rainy season (Tables 1-3). The houses positive for aegypti larvae increased from 24% during the dry season to 43% in the early rainy season.

The seasonal prevalence of aegypti larvae and pupae inside and outside of the 3 different basic types of residences is summarized in Tables 4-5. These data demonstrate an increase in aegypti positive containers both inside and outside houses, between the hot and the early rainy seasons. The slum residences yielded the highest number of aegypti positive containers and positive residences during both seasons. However, shop-house residences had the highest percentage of inside positivity for aegypti during the early wet season. These data suggest that the slum residences maintain the largest reservoir of aegypti through the dry season.

Estimate of the population density of Ae. aegypti

A standardized water (ong) jar was placed in the residence for each of the 100 study families. These jars will serve as larval habitats for aegypti, and thus provide mosquitoes for population density estimates in the study residences. A number of mosquito population sampling devices are being developed for use in the study area. These devices have been designed to eliminate the human sampling bias associated with most of the current techniques used for estimating population density of aegypti.

An inexpensive larval trap designed to float on the water surface in water jars has been developed and tested. This circular trap is made of clear plastic and measures approximately 13 cm in diameter and 13-14 cm in depth. The trap is designed so that larvae moving vertically toward the surface of the water are captured in a container after passing up through an inverted funnel. Data (Table 6 and 7) suggest that this trap is a highly efficient sampling method for Ae. aegypti and Culex quinquefasciatus larvae and/or pupae. These traps are currently being used to estimate population densities for aegypti in selected houses in the study area.

An emergence trap, a trap for ovipositing females and a battery operated suction trap for adults have been developed and partially tested. Data accrued to date suggest that all 3 are good sampling devices and will be satisfactory for estimating the population density of aegypti. The emergence traps are currently employed in the study area.

Seasonal assessment of the vector capabilities of Ae. aegypti, and genetic studies of aegypti

Techniques are being developed for determining the vector capabilities of aegypti during the different seasons in Bangkok. Preliminary results indicated that A. aegypti became infected after feeding on droplets of blood containing dengue virus and 10% sucrose. However, transmission of dengue viruses could not be demonstrated after a period of up to 30 days following ingestion of the viruses.

Studies are also being conducted to determine the seasonal esterase profiles of aegypti from the dengue virus ecology study area. In addition, esterase profiles are being determined for several laboratory colonies of aegypti. The methodology for these studies is based on electrophoresis starch-gel techniques described by Steiner and Joslyn (in press). Currently, there are 4 colony strains of Thai aegypti being tested. Strains that exhibit distinct esterase polymorphisms will be tested for susceptibility to infection with dengue viruses.

A total of 22 enzymes have been tested against 50 larvae each of 3 colony strains of aegypti and a total of 6 esterases were detected although most colonies exhibited only 4 esterases. The relative frequencies of esterase alleles at the Est-1 and Est-2 loci in larvae of 3 strains of aegypti and 2 closely related Stegomyia species, are shown in Table 8. These data demonstrate the ability of electrophoresis techniques to detect esterase and esterase allele differences between closely related strains of aegypti and closely related Aedes (Stegomyia) species. Data for the other 4 esterases detected in the colony strains are being analyzed.

Transovarial transmission of dengue viruses by Ae. aegypti

Aedes aegypti larvae are being collected from the residential premises of dengue hemorrhagic fever patients in Bangkok, Thailand. Larvae are being assayed for dengue viruses by the mosquito innoculation technique emplying Toxorhynchites splendens and by plaque assay employing LLC-MK_2 cell cultures.

A total of 531 pools consisting of 13,005 aegypti larvae were collected from 50 premises during the period November 1977 - March 1978. Thus far, attempts to isolate dengue viruses from 172 pools (4,280 larvae) have been unsuccessful.

(Douglas M. Watts, Bruce A. Harrison and David E. Johnson)

(We gratefully acknowledge the assistance of Dr. W.W.M. Steiner, Univ. of Illinois, with the electrophoresis studies).

Table 1. Seasonal Utilization of Containers for 100 Houses by Aedes aegypti Larvae and/or Pupae, Din Daeng, Bangkok, Thailand

Date of Surveys	Containers with Water				Percentage of Containers with A. aegypti		Percentage of Houses Positive for A. aegypti
	Larvae and/or Pupae		No Larvae or Pupae				
	Inside	Outside	Inside	Outside	Inside	Outside	
3-18 April, 1978 (Dry Season)	55	11	532	68	9.4(55/587)	13.9(11/79)	29
30May - 15 June (Early Wet Season)	86	24	489	72	15.0(86/575)	25.0(24/96)	43

Table 2. Seasonal Change in Utilization of Containers Inside 100 Family Dwellings* by Aedes aegypti Larvae and/or Pupae, Din Daeng, Bangkok, Thailand

Date of Survey	A. aegypti Larvae and/or Pupae			% Change (±) from Dry Season		
	Containers	Dwellings	Container/Dwelling	Containers	Dwellings	Container/Dwelling
3-18 April 78 (Dry Season)	55	25	2.20	-	-	-
30 May-15 June 78 (Early Wet Season)	86	37	2.32	+56	+48	+5

Table 3. Seasonal Change In Utilization of Containers Outside 100 Family Dwellings* by Aedes aegypti Larvae and/or Pupae, Din Daeng, Bangkok, Thailand

Date of Sruvey	A. aegypti Larvae and/or Pupae			% Change (±) from Dry Season		
	Containers	Dwellings	Container/Dwelling	Containers	Dwellings	Container/Dwelling
3-18 April, 78 (Dry Season)	11	8	1.37	-	-	-
30 May - 15June,78 (Early Wet Season)	24	14	1.71	+118	+75	+25

*Same family dwellings used during both surveys

Table 4. The Seasonal Utilization of Containers Inside 100 Houses by Aedes aegypti Larvae and/or Pupae for Different Types of Residences and the Number of Larvae and/or Pupae Pooled for Virus Isolation Studies*

Residential Type	# of Units	Dry Season			Early Wet Season		
		Positive for aegypti			Positive for aegypti		
		Containers	Units	Cont./Unit	Containers	Units	Cont./Unit
Highrise Flats	44	9	2	4.50	15	6	2.50
Slums	36	33	15	2.20	40	18	2.22
Shop or House	20	13	8	1.63	31	13	2.38
Total	100	55	25	2.20	86	37	2.32

Table 5. The Seasonal Utilization of Containers Outside 100 Houses by Aedes aegypti Larvae and/or Pupae for Different Types of Residences and the Number of Larvae and/or Pupae Pooled for Virus Isolation Studies*

Residential Type	# of Units	Dry Season			Early Wet Season		
		Positive for aegypti			Positive for aegypti		
		Containers	Units	Cont./Unit	Containers	Units	Cont./Unit
Highrise Flats	44	0	0	0	0	0	0
Slums	36	10	7	1.43	19	12	1.58
Shop or House	20	1	1	1.00	5	2	2.50
Total	100	11	8	1.37	24	14	1.71

* The same family units used during both surveys.

Table 6. Aedes aegypti Larvae Captured in Traps During a 24 Hour Period

Trials*	Date Test Ends	Larvae Trapped Per Container					Captured	
		Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Total	%
1	4 Aug	24	56	61	70	48	259	51.8
2	8 Aug	31	59	39	18	40	187	37.4
3	11Aug	57	74	80	67	67	345	69.0
4	16Aug	69	61	58	56	23	267	53.4
5	18Aug	67	64	72	45	62	310	62.0
Total		248	314	310	256	240	1,368	54.7

Table 7. Culex quinquefasciatus Larvae Captured in Traps During 24 Hour Period

Trials*	Date Test Ends	Larvae Trapped Per Container					Captured	
		Trap 1	Trap 2	Trap 3	Trap 4	Trap 5	Total	%
1	4 Aug	71	54	62	74	24	285	57.0
2	8 Aug	47	30	71	67	64	279	55.8
3	11 Aug	56	27	52	88	60	283	56.6
4	16 Aug	62	40	84	75	85	346	69.2
5	18 Aug	65	76	65	43	60	309	61.8
Total		301	227	334	347	293	1,502	60.1

* Each trial tested 5 replicates, each involving one trap per each 27 liter water jar and 100 fourth stage larvae per jar.

Table 8. Relative frequencies of esterase alleles at the Est-1 and Est-2 Loci in larvae of 3 colonies of Aedes aegypti and colonies of Aedes albopictus and Ae. malayensis.

Locus	Mosquito Colonies-Strains	Allele					
		.92	.94	.96	.98	1.00	1.02
Est - 1	aegypti - 1	-	-	-	-	.70	.30
	aegypti - 3	-	-	-	-	.89	.11
	aegypti - 4	-	-	-	.53	.43	.03
	albopictus-2	-	-	.02	.39	.59	-
	malayensis-1	.30	.40	.30	-	-	-
Est - 2	aegypti - 1	-	-	-	1.00	-	-
	aegypti - 3	-	-	-	-	1.00	-
	aegypti - 4[1]	-	-	-	-	Null?	-
	albopictus-2[2]						
	malayensis-1[2]						

1 This colony has the Est-1, .98 allele overlapping the Est-2 zone of activity, making it difficult to assess the presence of no activity.

2 Enzyme activity too low to accurately diagnose the banding patterns.

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One way of controlling dengue virus transmission is to control the mosquito vectors. Aedes aegypti and Aedes albopictus are proven to be the efficient vectors for dengue viruses in Thailand. Mosquito control today is in a state of crisis because mosquito resistance to chemical pesticides has caused the failure of many vector control campaigns especially in the campaign to prevent the transmission of dengue viruses.

Bacillus thuringiensis strain HD-1 and BA-068 have been shown to produce the parasporal protein and this protein is toxic to Aedes aegypti larvae following the feeding of the larvae. The toxicity of B. thuringiensis HD-1 strain varied slightly when tested in different types of mosquito larvae. The LC_{50}'s of B. thuringiensis toward Aedes aegypti, Culex quinquefasciatus and Anopheles balabacensis were found to be 1.2×10^5, 8.2×10^5 and 1.1×10^4 organisms/ml respectively.

The toxic factor was believed to associated with the crystalline body of the bacterium since a sporogenic but acrystalliferous mutant (strain HD-S) of B. thuringiensis, HD-1, was found to lack the ability to kill mosquito larvae. A revertant (strain HD-SR) of strain HD-S which possessed crystal was selected and was found to have regained the ability to kill larvae. Also, an oligosporons but crystalliforous mutant (strain R-201) selected from rifamycin resistant strains of B. thuringiensis, HD-1, demonstrated the same level of toxicity toward Ae. aegypti larvae as the wild type. Cultures of this mutant contained one thousand fold fewer spores than cultures of the wild type but contained the same quantity of crystals.

In addition, the ability of Toxorhynchites splendens larvae to destroy Ae. aegypti larvae in the amount of 20-25 larvae/day. The larvae of Toxorhynchites splendens were released into the water containers or breeding sites of Ae. aegypti, Ae. albopictus in the study area in the amount of 10-100 larvae per site depends on the size of the container or breeding site. The larvae of Ae. aegypti and Ae. albopictus were eliminated completely or almost completely in 3-4 days. Then B. thuringiensis was applied into those containers or breeding sites to control the newly emerged larvae. The experiment is still going on.

(Somsak Pantuwatana, Amaret Bhumiratana, Wiwit Samasanti, Penchit Premabutr)

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

Dengue-2 infection in mice after passive transfer anti-dengue-1 serum.

In a previous communication it was shown that intracerebral injection of 10 LD_{50} dose of adult-adapted dengue type-2 (AD_2) in mice which received intraperitoneal injection of dengue type-1 (D_1) one, two, three or four weeks previously produced microscopic (occassionally macroscopic) haemorrhage in some of the internal organs (liver, lungs, heart, kidney and brain), and reduced significantly the platelet count of the doubly infected mice.

To find out the effect of dengue-2 infection of mice which received passive transfer of anti D_1 serum, the following experiment was conducted :

Three groups (A, B, and C) of 4-6 weeks old mice were injected subcutaneously with 0.2 ml of anti-dengue-1 mouse serum. The serum was collected 7 days after one, two or four intraperitoneal injections of 10 LD_{50} of dengue type 1 virus. Group A was challenged intracerebrally(I.C) with 10 LD_{50} of AD-2, 24 hours after receipt of serum, Group B, 48 hours after, and Group C, 7 days after. Another group (Gr.D) without previous receipt of serum was challenged with 10 LD_{50} of AD-2, to serve as control. The dose of the anti-dengue serum and the interval between injection of serum and challenge by AD-2 were empirical. When the mice became ill, they were sacrificed after doing platelet count and bleeding time(BT). During autopsy examination search for macroscopical haemorrhage in organs was made. Histological examination for microscopical haemorrhage was made in heart, lungs, brain, liver and kidney.

Preliminary results of examination are as shown in the Table below:

Expt. No.	Anti D-1 Serum after	Mouse Group	BT (in minutes)	Platelet (in log per c.mm)	Microscopical haemorrhage present in
1.	4 injections (HI titre 1:40	A	5.00	5.18	Liver,Lung.
		B	5.00	6.29	Lung.
		C	4.5	5.28	Heart,Lung,Liver.
		D(Control)	3.9	5.24	Lung.
2.	1 injection (HI:- ⟨10)	A	4.45	5.08	Lung.
		B	5.25	4.86	Liver,Lung,Kidney.
		C	5.10	5.09	Lung.
		D	4.30	5.26	NIL
3.	2 injections (HI:- 1:20)	A	ND	5.90	Kidney,Liver.
		B	ND	5.91	Kidney,Lung.
		C	ND	6.04	Kidney,Liver.
		D	ND	5.20	NIL

(J.K.Sarkar, A.C.Mitra, K.K.Mukherjee, S.K.Chakravarty, M.S.Chakravarty, & K.K.Mitra)

SUSPECTED JAPANESE ENCEPHALITIS EPIDEMIC IN BANGLADESH IN 1977

An epidemic of encephalitis occured in August 1977 mostly amongst the tribal people (Garo tribe) of the Mymensingh district of Bangladesh. The villages affected were Bagadoba Jalchatra, Shainamari, Bhutla, Rajabari, Thelki and Bhutia in the Garo hill area of Bangladesh. The acute and convalescent samples of blood from suspected cases were collected and sent to the department of Virology, School of Tropical Medicine, Calcutta by the World Health Organisation(WHO) authorities at Dacca, Bangladesh for serodiagnosis. The serum samples were tested for presence of both Haemagglutination Inhibition (HI) and Complement fixing (CF) antibodies against both Group A and Group B arboviruses. The serum samples having antibody against Japanese encephalitis(JE) virus were further analysed qualitatively for IgM antibody.

The results of 3 paired and a few representative single serum samples are presented in Table I and Table II.

Table-I

Results of serological testing of paired serum samples against Group B arboviruses:

Case No.	Day of illness	HI titre C	HI titre D	HI titre JE	HI titre WN	CF titre C	CF titre D	CF titre JE	CF titre WN
1. 9 yrs. Female	5 days	<10	20	80	80	<4	<4	<4	<4
	25 days	<10	40	320	160	<4	<4	8	4
2. 10 yrs. Male	7 days	<10	10	80	80	<4	<4	<4	<4
	27 days	<10	20	320	320	<4	<4	16	4
3. 35 yrs. Female	6 days	<10	20	40	80	<4	<4	<4	<4
	26 days	<10	40	160	160	<4	<4	16	8

Table-II

Results of testing of representative single serum samples against Group B arboviruses:

Case No.	Day of illness	HI titre				CF titre				IgM antibody testing.
		C	D	JE	WN	C	D	JE	WN	
7	18	<10	<10	80	20	<4	<4	32	4	Positive
24	12	<10	<10	160	80	<4	<4	16	4	Positive
23	16	<10	40	160	160	<4	8	16	16	Negative
28	11	<10	320	>640	>640	<4	8	8	8	Negative
45	7	<10	10	<10	<10	<4	<4	<4	<4	Negative

C : Chikungunya
D : Dengue
JE : Japanese Encephalitis
WN : West Nile

The clinical symptomatology as provided by the WHO authorities in Bangladesh, the seasonal outbreak(the month of August being the monsoon season of the area), and results of serological investigations strongly suggest that the epidemic of encephalitis was due to JE virus, Possibly this is the first report of JE epidemic in Bangladesh.

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REPORT FROM ARBOVIRUS UNIT
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Isolation of Tick-borne encephalitis virus from ticks in Italy.

In Toscana region (Central Italy) cases of tick-borne encephalitis were diagnosed in the past (see Arbo.Info. Exchange n.30, pag. 176, March 1976). Field studies were then undertaken to find out whether natural foci of tick-borne encephalitis (TBE) virus were present in this part of the country.

Ticks were collected both on grass and from domestic animals in two localities of Toscana region (Southern and Northern) where infections of patients were suspected to have occurred. Tick collections were performed from June 1977 to December 1978 mainly in the Southern locality. The species most commonly found were Ixodes ricinus, Rhiphicephalus sanguineus and Rhiphicephalus bursa (Table 1)

Small rodents, namely 140 Apodemus sylvaticus, 23 Cletrionomys glareolus and 12 Mus musculus, were caught into the live-trap in one locality (Southern) from June 1977 to October 1978.

Suspensions were prepared from ticks and from organs (brain, liver, spleen and kidney) of small rodents. Isola= tion experiments were done by i.c. inoculation of 1-2 days old suckling mice with 0.01 ml of suspension. One virus strain (ISS.IR.968) was isolated from a pool of 14 females of Ixodes ricinus ticks collected on 29 May 1978 in the Northern locality. The incubation period in the 1st mouse passage was 5-6 days; this was shortened to 3-4 days in

subsequent mouse passages. The virus was reisolated from the original suspension kept at -70 C. The virus was pathogenic for weanling mice by i.c. and i.p. inoculation. A hemagglu= tinating antigen was prepared from infected mouse brains by Arcton extraction. Optimal agglutination occurred at pH 6.6 at 37 C with a titer of 1:320.

The virus was identified as closely related to TBE virus (Hypr strain) by CF, HI and neutralization (in i.p. inocula= ted weanling mice) tests (Table 2). TBE horse antiserum, kindly supplied by Dr. M. Gresikova (WHO Collaborating Centre for Arbovirus reference and research, Bratislava) and mouse immune ascitic fluid prepared to the newly isolated virus strain were used in the tests.

No virus was isolated from ticks and mammals collected in the Southern locality of the region. This is the first isolation of TBE virus in Italy, although its circulation was suspected on the basis of previous serological studies. No work with TBE virus was performed in our laboratory since the beginning of isolation experiments in June 1977.

Sera from sheep grazing in the two localities were tested for antibodies to TBE virus by HI test. None out of 60 sheep sera from Southern locality was positive, while 6 out of 60 sheep sera from Northern locality had antibodies to TBE antigen.

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Table 1 - Number and stages of ticks processed for virus isolation in two localities of Toscana region.

Ticks		Locality	
		Southern	Northern
Ixodes ricinus	Nymphs	27	32
	Males	242	10
	Females	356	28[+]
	Total	625	70
Rhiphicephalus spp.	Nymphs	13	130
	Males	871	250
	Females	358	211
	Total	1,242	591
	Totals	1,867	661

[+]Isolation of TBE virus

Table 2 - Identification of TBE virus strain (ISS.IR.968) by CF, HI and N tests.

	Serum or ascitic fluid				
	ISS.IR.968	TBE	ISS.IR.968	TBE	TBE
Antigen or virus	CF		HI		N
ISS.IR.968	32/8[+]	32/16	80[++]	1280	5.7[+++]
TBE	32/16	32/32	80	2560	not done

[+] serum titer/antigen titer

[++] reciprocal of serum dilution

[+++] NI_{50}

REPORT FROM THE NATIONAL INSTITUTE OF HYGIENE BUDAPEST, HUNGARY

In collaboration with the Institute of Virology, Slovak Academy of Sciences, Bratislava, Czechoslovakia, investigation of different TBE natural foci was carried out in the Western part of Hungary, in 1970, 1972 and 1977. In addition to the 12 TBE virus strains reported previously 3 Tettnang, 1 Uukuniemi and 2 arbovirus strains belonging to the Congo group have been isolated. Virus isolation experiments were performed by intracerebral inoculation of suckling mice and identifications by HI and CF tests. Of the TET virus strains one was isolated from the heparinized blood sample of a C.glareolus captured in 1970, another one from I.ricinus nymphae and the third one from D.reticulatus adults collected in 1977. The UUK virus strain was isolated from I.ricinus females collected in 1972. In the same year, 2 further arbovirus strains were isolated from I.ricinus nymphae. Sucrose-acetone antigen prepared from the brain of infected suckling mice fixed the complement in presence of CON antiserum.

/E.Molnár, J.Nosek, O.Kozuch, E.Ernek, M.Labuda, M.Gresiková/

REPORT FROM THE INSTITUTE OF VIROLOGY, SLOVAK
ACADEMY OF SCIENCES, BRATISLAVA
CZECHOSLOVAKIA

Reaction of the host to the bite of viruliferous Ixodes ricinus females.

No distinct internal cement formation was observed at the penetration site of the mouthparts of Ixodes ricinus females during their feeding on golden hamsters. At 2 h. the chelicera were already deeply inserted into the dermal tissue. Beginning after 4 hours, the proliferation of collagen and conus formation became evident. The internal cell-free area was structureless and showed PAS positivity, while the outer collagen layer was rich in fibrocytes and negative for PAS staining. Both layers showed positivity when stained for collagen. The conus extending into the subdermal connective tissue was surrounded by an infiltrate of mononuclear and polynuclear leukocytes. The half-engorged females showed specific fluorescence of the TBE antigen in columnar epidermal cells. The penetration of the TBE virus into host tissue lasted probably only for a few h, as virus was succesfully transmitted to golden hamsters during 2 h feeding of the viruliferous I. ricinus females.

/J.Nosek, J.Rajčáni and J.Kožuch/

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

Transovarial transmission of Ťahyňa virus by Aedes vexans mosquito and the proof of its role as hibernation mechanism

Since the first description of transovarial transmission of La Crosse virus by Aedes triseriatus mosquitoes (Watts et al., 1973) vertical transmission has been found with other members of the group of California encephalitis virus on North-American continent (Le Duc et al., 1975, Mc Lean et al., 1975, Andrews et al., 1977). The transovarial transmission may also play a role of hibernation mechanism.

The Ťahyňa virus was isolated from larvae of Culiseta annulata mosquito which suggests that the transovarial transmission was involved (Bárdoš et al., 1975). Since this mosquito overwinters as imago, the transovarial transmission can not play a role of hibernation of the virus. Nevertheless, till now we have considered this species important for virus hibernation with respect to the experimental proof of virus Ťahyňa hibernation in this mosquito under natural conditions (Danielová & Minář, 1969). However all ecological observations suggest that Aedes vexans, which is considered a dominant vector in lowland forest natural foci of Central Europe, gives rise to the summer explosion of Ťahyňa virus circulation.

Since the field minimal infection rate of this mosquito species is rather low, we made a laboratory experiment to prove the transovarial transmission of Ťahyňa virus by Aedes vexans.

Aedes vexans females were collected wild and were infected by feeding on viremic hamsters; viremic blood on cotton-wool was exposed next day to those who did not feed during the supposed peak of hamsters viremia. The viremia was induced by intramuscular inoculation of extraneurally passaged strain 236 of Ťahyňa virus 69 - 73 hours before mosquito feeding. Immediately after exposure to mosquitoes, the hamsters blood was separately titrated and stored in mixture at -30° C to the next day when it was exposed to unfed mosquitoes.

Engorged mosquitoes were transferred to new cages and separated according to hamsters upon which they fed. The viremia reached 1,5 log $TCID_{50}$ of virus only in one of six hamsters, 4,0 - 4,5 log $TCID_{50}$ in the others and 4,17 in mixture blood fed on the next day. Eggs of all these mos-

quitoes were therefore processed together. The mosquitoes after infection feeding were given 10% glucose and water and several times another blood to obtain as many eggs as possible. The eggs were divided into five groups according to the period of oviposition after mosquito infection without respect to ovarial cycle of females as we suppose that the interval after infection influences virus penetration to ovaria (Danielová, 1968). After intervals when embryogenesis was anticipated to be passed (17 - 30 days after oviposition, the last group after 41 - 44 days) the eggs were left to hatch. The eggs which did not hatch were stored in a refrigerator from the autumn to the next spring. They were then transferred to room temperature and a high humidity for one month and subsequently they were left to hatch.

In autumn when the eggs were laid, 110 larvae, 234 females, and 213 males of F_1 generation were processed and virologically tested in 47 pools while in the next spring 33 larvae, 90 females, and 94 males were tested in 18 pools. No virus was isolated from F_1 mosquitoes processed in autumn (September - October) while from F_1 mosquitoes processed in spring (May) 4 strains of Tahyňa virus were isolated. One of them was isolated from a pool of females, three from pools of males. Two virus isolations were from F_1 mosquitoes from an oviposition 25 - 32 days after infection of parent females and one from F_1 mosquitoes from eggs laid 21 - 29 and one 13 - 18 days after infection of females. No virus was isolated from F_1 mosquitoes hatched from eggs laid by 12 days after infection of parent females.

As no laboratory of our department worked with Tahyňa virus at the time of virus isolation (for the last time it was in previous year when parent females were infected) we suppose that transovarial transmission of Tahyna virus by Aedes vexans mosquitoes, as well as the virus hibernation by this mechanism, are undoubtedly proved. This fact explains all our ecological observations in which the virus was detected after emerging of Aedes vaxans mosquitoes in natural focus where it acts as a dominant vector.

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V. Danielová and J. Ryba

Some biological and antigenic characteristics of Ťahyňa virus strains isolated from Culiseta annulata /Schrk./ larvae and from blood of sick children.

Ťahyňa virus was isolated in 1974 from field collected Culiseta annulata larvae and from blood of two sick children /1,2/ in southern Moravia /Czechoslovakia/. Since this locality is 380 km beeline from eastern Slovakia /Czechoslovakia/, where the prototype strains of Ťahyňa virus had been isolated in 1958, we have decided to compare these strains.

For the present study two criteria were chosen, namely the size of plaques and the antigenic interrelationship.

The size of plaques was determined after 72 hours in Vero cells. All strains were simultaneously tested in one test. Table 1 demonstrates the strains under study. Table 2 and 3 are showing the size of plaques and the significance of differences.

The antigenic interrelationship /R/ was first tested in a PRNT in Vero cells with 6 week rabbit immune sera (Tables 4,5 and 6) and later with 10 day rabbit immune sera /Tables 7 and 8/.

The results of the later test have confirmed that there are antigenic differences between the strains isolated in 1958 and 1974 as well as between the strains LA 65 isolated from Culiseta annulata larvae and the strains T 16 and P 6b isolated from blood of sick children in 1974.

The antigenic interrelationship was tested also in a counter immunoelectrophoretic test. The minimal concentration of antigens for optimal production of a distinct precipitating line was

determined by an optimal proportion titration /"grid"titration/. The 10 day immune rabbit sera were tested with 2-4 units of antigens. Results are seen in the Table 9. The calculated "R" values are seen in the Table 10.

1. V.Bárdoš, J.Ryba, Z.Hubálek, Acta virol.19:446, 1975
2. V.Bárdoš, M.Medek, V.Kania, Z.Hubálek, Acta virol.19:447,1975

(V.Bárdoš, J.Peško)

Prevalence of Ťahyňa virus-neutralizing antibodies among patients in southern Moravia, Czechoslovakia: 1973-76

Three groups of persons (inhabitants of Břeclav district) were tested for prevalence of antibodies to Ťahyňa (TAH) virus, (California group) by a modified plaque-reduction neutralization test (PRNT) in PS cells.(1).

Group I: 117 children aged 0-15 years, both sexes, suffering from acute febrile illness. Their blood samples were collected in the period March till October, 1973-76.

Group II: 27 adult patients of both sexes, hospitalized under diverse diagnoses. The blood samples were collected in April till October, 1974-76.

Group III: 47 gravid women, without febrile illness. Their blood samples were collected in March till December, 1973-74.

PRNT titers (50 % plaque reduction) distribution among the persons:

Titer:	< 1:4	1:4	1:8	1:16	1:32	1:64	1:128
No. of persons:	75	9	1	1	5	3	8

1:256	1:512	1:1024	1:2048	1:4096
20	35	24	9	1

From the total number of persons tested (191), 55.5 % had neutralizing antibodies to TAH virus (the titer $>$ 1:8). GMT in the positive sera was 1:435.

Age groups: Mean age of the persons was 20 years. The prevalence of antibodies was significantly correlated with the age:

Age groups (yrs.):	<3	3-4	5-6	7-8	9-12	13-18	19-24	25-30	31-70
Persons examined:	18	12	26	26	23	16	33	16	21
Persons positive (%):	5,6	8,3	46,2	53,8	56,5	62,5	75,8	81,3	81,0

GMT in the persons aged 18 years or less was 1:700, whereas in the older persons 1:280 ($P < 0,001$). The higher titers of antibodies in the younger group could be explained by the higher proportion of recent TAH infections in this group.

Sex. There was no difference in the prevalence and GMT of antibodies between males and females.

Locality: No significant difference in the prevalence of antibodies was found among the persons inhabiting various localities within the district. The whole area is known as a natural focus of TAH virus, with abundant population of mosquito vectors, Aedes vexans predominantly.

1. de Madrid A.T., J.S. Porterfield; Bul.W.H.O. 40: 113, 1969

(Z.Hubálek, V.Bárdoš, M.Medek[x], V.Kania, L.Kychler[x], E.Jelínek[x]).

[x]District Health Centre, Břeclav.

TABLE 1

SUMMARY OF PEDIGREES OF THE FIVE TAHYNA VIRUS STRAINS STUDIED

VIRUS STRAIN	ORIGIN	LOCALITY	YEAR OF ISOLATION	PASSAGE HISTORY
92	AEDES CASPIUS/IMAGO/	EASTERN SLOVAKIA	1958	YMB5[x]SMB3 YMB1 SMB2
236	AEDE VEXANS /IMAGO/	EASTERN SLOVAKIA	1958	HBL5[xx]RBL3 HBL1
LA 65	CULISETA ANNULATA/LARVAE/	SOUTHERN MORAVIA	1974	SMB2
T 16	BLOOD FROM A FEBRILE PATIENT	SOUTHERN MORAVIA	1974	SMB1
P6 b	BLOOD FROM A FEBRILE PATIENT	SOUTHERN MORAVIA	1974	SMB1

[x] YMB resp.SMB = YOUNG resp. SUCKLING MOUSE BRAIN

[xx] HBL resp.RBL = Hamster resp.rabbit blood

TABLE 2

COMPARISON OF PLAQUE SIZE DISTRIBUTION IN VERO CELLS (AFTER 72 HOURS)

VIRUS STRAINS	N° OF PLAQUES ASSAYED	RANGE (mm)	MEAN ± 1 SD (mm)	COEFFICIENT OF VARIATION (%)
92	90	0.25-0.75	0.4 ± 0.18	45.0
236	99	0.5 -1.4	0.8 ± 0.3	37.5
LA 65	62	0.6 -1.7	1.1 ± 0.4	36.3
T 16	106	0.4 -1.1	0.6 ± 0.16	26.7
P6 b	78	0.35-1.25	0.7 ± 0.27	38.6

TABLE 3

THE SIGNIFICANCE OF THE DIFFERENCES IN THE SIZE OF PLAQUES

(t)

VIRUS STRAINS

VIRUS STRAINS	92	236	LA 65	T 16	P6 b
92		10.0	14.0	10.0	10.0
236			5.0	6.7	2.5
LA 65				10.0	6.7
T 16					3.3
P6 b					

TABLE 4

DETAILS OF THE IMMUNIZATION SCHEDULE

IN PREPARING I. M. IMMUNE RABBITS SERA

STRAINS OF VIRUS	WEIGHT OF RABBITS IN GR.	INOCULATION DOSE IN DEX PFU	VIRUS DILUTED IN PBS	DAY OF BLEEDING
92	620.0	8.4	+ C.F.A.[x]	43
	870.0	5.1		10
236	750.0	2.2		9
LA 65	680.0	6.7	+ C.F.A.	44
	740.0	2.8		10
T 16	550.0	7.6	+ C.F.A.	41
	770.0	3.9		10
P6 b	800.0	7.2	+ C.F.A.	47
	990.0	3.7		10

[x]COMPLETE FREUND'S ADJUVANT

TABLE 5

RESULTS OF CROSS PRNT WITH 6 WEEKS I.M. IMMUNE RABBIT SERA

	ANTIGENS			
SERA	92	LA 65	T 16	P6 b
92	1024[x]	1024	256	256
LA 65	4096	8192	32768	4096
T 16	2048	2048	8192	4096
P6 b	2048	2048	32768	4096

[x] Reciprocal antibody titer

TABLE 6

INTERSTRAIN "R" VALUES (IN %) OBTAINED FROM THE PRNT WITH 6 WEEK I.M. IMMUNE RABBIT SERA

SERA	ANTIGENS			
	92	LA 65	T 16	P6 b
92	100.0	70.7	25.0	35.3
LA 65		100.0	100.0	50.0
T 16			100.0	200.0
P6 b				100.0

"R" = Relatedness

TABLE 7

RESULTS OF CROSS PRNT WITH 10 DAY I.M.
IMMUNE RABBIT SERA

	ANTIGENS				
SERA	92	236	LA 65	T 16	P6 b
92	<4	<4	<4	<4	<4
236	1024[x]	4096	4096	4096	4096
LA 65	512	128	8192	4096	512
T 16	64	256	512	1024	256
P6 b	256	64	256	1024	256

[x] Reciprocal antibody titer

TABLE 8

INTERSTRAIN "R" VALUES (IN %) OBTAINED FROM THE PRNT
WITH 10 DAY I.M. IMMUNE RABBIT SERA

	ANTIGENS				
SERA	92	236	LA 65	T 16	P6 b
92	0.0	0.0	0.0	0.0	0.0
236		100.0	12.5	50.0	25.0
LA 65			100.0	50.0	25.0
T 16				100.0	100.0
P6 b					100.0

TABLE 9

CROSS COUNTERCURRENT IMMUNOELECTROPHORESIS TEST WITH 10 DAY I.M. IMMUNE RABBIT SERA

SERA	ANTIGENS				
	92 (1:8)[x]	236 (1:16)	LA 65 (1:32)	P6 b (1:16)	T 16 (1:4)
92	0	0	0	0	0
236	16[xx]	32	16	32	8
LA 65	8	8	8	8	8
P6 b	16	32	16	32	8
T 16	4	4	2	2	4

[x] 2 UNITS OF ANTIGEN AT A DILUTION OF 1:8

[xx] RECIPROCAL TITRES

TABLE 10

INTERSTRAIN "R" VALUES (IN %) OBTAINED IN THE CEP WITH 10 DAY I.M. RABBIT IMMUNE SERA

SERA	ANTIGENS				
	92	236	LA 65	T 16	P6 b
92	0.0	0.0	0.0	0.0	0.0
236		100.0	70.7	50.0	100.0
LA 65			100.0	70.7	70.7
T 16				100.0	35.3
P6 b					100.0

REPORT FROM THE DEPARTMENT OF VIROLOGY, VACCINATION CENTER (LANDESIMPFANSTALT), AUF'M HENNEKAMP 50, D-4000 DUESSELDORF, WEST GERMANY

In 1972 there was built a new institute in Duesseldorf, West Germany, mainly for the production of smallpox vaccines. But the concept was that this institute should change into a tropical institute when smallpox would be eradicated in the world. As this was the case in 1978 the production of smallpox vaccines was reduced to a low level and it was started with a training of the staff in tropical diseases, mainly virus diseases.

The main task of the Department of Virology will be the diagnostic of tropical virus diseases. Dr.JUERGEN PILASKI, the chief virologist of this department, has been trained for three months during 1978 by the Yale Arbovirus Research Unit at New Haven and has been supplied with about 65 arbovirus strains by this institute. In the next months the staff of the Department of Virology will be engaged in preparing antigens and antisera for diagnostic.

There are several research projects running or being started together with medical students of the University of Duesseldorf:

1) ANDREAS SCHWARTZ: Inactivation of denguevirus by heat, formaline and betapropiolactone.

2) ANNETTE GREWE: Serological survey on arbovirus infections in humans and animals in Uganda and Northern Kenya.

3) FRANK NELLES: Pathogenesis of Tahyna virus in different species of Culicidae.

The results of these projects will be submitted to this information exchange as soon as they are available.

(Jürgen Pilaski)

REPORT FROM THE FEDERAL RESEARCH INSTITUTE FOR ANIMAL VIRUS DISEASES, TÜBINGEN, FEDERAL REPUBLIC OF GERMANY

MICRO TISSUE CULTURE FOR DIAGNOSIS OF TOGAVIRUSES BY INDIRECT IMMUNOFLUORESCENCE

Methods were developed for the quantitative assay of selected Togaviruses with the aid of the fluorescent antibody technique. The following viruses were used in the test: Sindbis and Semliki Forest viruses from the genus Alphavirus, West Nile virus from the genus Flavivirus, and Hog Cholera virus from the genus Pestivirus.

A suspension of BHK cells (in the case of Hog Cholera virus, PK-15 cells were used) was prepared from monolayer cultures and infected subsequently. The culture (2 ml) containing usually $1x10^6$ cells per ml Medium (MEM complemented with 5% fetal calf serum) was stirred for 12 - 24 hours depending on the virus used for infection, with a magnetic stirrer at 37°C. After incubation, the suspension was adjusted to a concentration of 10^4 cells per ml. Aliquots of 0.2 ml were centrifuged onto microscope slides (3 min at 1,500 rpm, Cytospin, Shandon-Elliot) and the slides were rinsed for 5 min with PBS followed by two washings in acetone and staining as usual.

In the microscopic picture the cells appeared somewhat flattened, nearly round and sometimes in clusters from 2 to 10 cells. It was possible to distinguish between nucleus and cytoplasm of the centrifuged cells. The use of this microtissue culture method in the fluorescent antibody technique allows the detection of virus with only about 10^3 cells for each determination. The titration of unknown viruses against several immune sera for diagnosis is therefore possible with a minimum of cells.

(P.J. Enzmann)

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

Studies on Soldado virus in France

I - Isolation of Soldado (SOL) virus in France (Cap Fréhel, Brittany)

In April 1977, eight strains of an apparently identical virus were isolated from 92 Ornithodoros (A.) maritimus Vermeil et Marguet, 1967, collected by Pr J.C. BEAUCOURNU (Rennes University) in nests of herring gulls Larus argentatus, on Cap Fréhel, Côtes-du-Nord (48° 41' N - 2° 19' W, *Map*), One strain was isolated from a pool of 5 o and the others from seven pools of O. All the strains were reisolated from the original material kept at -70°C.

The strains were pathogenic for suckling and adult mice by the intracerebral but not by the intraperitoneal route, inducing acute encephalomyelitis and death. No hemagglutinin was demonstrated. Attempts to adapt these strains to tissue culture, including BHK 21 and RK 13, were unsuccessfull.

The Brest/Ar/T 13 strain was more completely investigated. This virus was highly sensitive to ethyl-ether and heat inactivation (1 hour at +60°C) but was stable at pH 3,0. By cross CF tests performed by Or J. CASALS at the Y.A.R.U., and by us with immune ascitif fluids received from Y.A.R.U., all the strains were found closely related to each other and to SOL virus (strain TR 52 214). Moreover, cross N tests performed with anti-SOL ascitic fluid and anti-T 13 immune serum showed that Brest/Ar/T 13 was closely related if not identical to SOL virus, though antibody against T 13 neutralized less efficiently SOL virus than Brest/Ar/T 13 *(Table 1)*.

In June 1978, Pr J.C. BEAUCOURNU collected again argasid ticks on Cap Fréhel, from cormorants (Phalacrocorax aristotelis), from herring gulls (L. argentatus) and from their nests. Four strains of viruses were isolated from 163 O. (A.) maritimus. Preliminary results indicated that these strains are very similar to those isolated in 1977 : their identification is in progress in our laboratory.

Interesting enough is the fact that the 1978 isolates originated from two points of collection where many young gulls were found dead.

II - Morphology and morphogenesis of Soldado virus : a proposal for incorporating the Hughes serogroup in the Bunyaviridae family of arboviruses

Electron microscope studies of the brain of moribund infant mice were performed for Brest/Ar/T 13 and Brest/Ar/T 14 strains, in comparizon with SOL virus, reference strain (TR 52 214).

Aggregates of extracellular virions were seen in all the sections of brain but mainly in the midbrain where inflammatory lesions were prominent. Virus particles were pleomorphic, generally round or ovale, rarely elongated. They had a mean diameter of 94 nm with extremes of 70 and 107 nm. They were delineated by trilamellar membrane 8-9 nm thick, with a fuzzy surface and bearing occasionnaly distinct "spikes". The internal density of virus particles varied from nearly lucent to moderatly dense, but it was always reinforced underneath the membrane.

The maturation of virions was seen occuring into distinded cisternae of Golgi apparatus and endoplasmic reticulum of neurons. The morphology of intracellular particles were quite the same as for extracellular ones, but their size was frequently smaller. Apparently mature virus particles were released from the infected cells by progressive necrosis and detachment of the Golgi apparatus.

The morphology and the morphogenesis of SOL virus, reference strain, were quite similar to those of the French strains.

So both morphology and morphogenesis of Hughes group viruses are very similar to those of Bunyaviruses and it seems legitime to propose the inclusion of this group of arboviruses in the Bunyaviridae family.

(To be published in "Comptes-Rendus Hebd. Acad. Sciences, Paris" and in "Archives of Virology").

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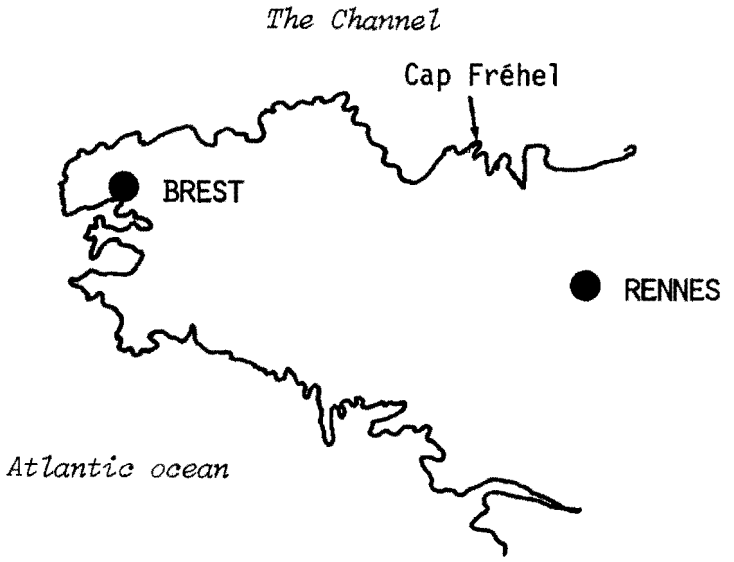

MAP OF BRITTANY

Antibody / Virus	IMMUNE ASCITIC FLUID anti-SOL (Y.A.R.U.)	IMMUNE SERUM anti-Brest/Ar/T 13 (BREST)
S O L	2 . 1	1 . 6
Brest/Ar/T 13	2 . 0	2 . 9

Table 1 - Results of cross N tests between SOL virus and Brest/Ar/T 13 virus (one-day old mice, intracerebral route).

MICROBIOLOGICAL RESEARCH ESTABLISHMENT
PORTON, SALISBURY, U.K.

The use of the Host-response phase-diagram in the in-vivo differentiation of Strains of Yellow Fever Virus

The strains of YFV used in this study (Table 1) are representative of isolations made since 1927 in Africa and the Americas. Strains were obtained at the lowest pass level available from Dr. J. Casals of YARU (Code Y) or Dr. Y. Robin of the Institut Pasteur de Dakar (Code D). Working stocks were produced in each case by a single additional pass in the brains of suckling mice. Estimations of virus infectivity were by conventional methods of plaque assay or titration of suckling mouse i.c. LD50. The plaque forming unit (p.f.u.) in agar suspensions of primary chick embryo cells or the suckling mouse i.c. LD50 unit are closely equivalent and will be defined together as the potentially infective unit. Serum neutralization indices (SNI) estimated by plaque reduction or ring-inhibition indicate the logarithm of the concentration or activity of specific antibodies.

Litters of A2G or Balb C mice of defined age (± 10% days) were used for the intraperitoneal titration of the YFV strain samples so that estimates were obtained for the number of infective units required for 50% death (LD50), 50% infection and protection in the absence of detectable antibody synthesis (P^-D50), and 50% protection in the presence of detectable antibody synthesis (P^+D50). In this estimation of protection the survivors to primary infection were bled for antibody assay on the 15th to 21st day and then challenged i.c. by 10^3 infective units of the 17D vaccine strain of YFV. These pre-challenge estimations of individual serum antibody activities all showed neutralization indices of 2.5 to 3.5 if positive or <1 SNI if negative: this demonstrated a sharp onset of vigorous antibody stimulation at a much higher dose than that required for infection.

The results in Figure 1 show the response-dose characteristics for LD50, P^+D50 and P^-D50 for the YFV strains Y1 (Asibi) (solid lines) and 17D (A) (broken lines) as detailed in Table 1. Although not shown in detail, each line is defined by about 10 individual titrations within 0.5 log units. The three broken lines for strain 17D (A) and the three solid lines for strain Y1 (Asibi) provide an in-vivo fingerprint or phase-diagram for each strain by which it may be differentiated in several ways.

Furthermore, these phase-diagrams show the independence and interaction of events on pathogenic and immunogenic pathways. Thus, for ages and doses above the LD50 lines in each case, the mice show CNS involvement and death with recovery of virus in brain at 10^7 to 10^9 infective units/brain. In age-dose zones below the LD50 lines the mice are protected and the earlier immunogenic stimulations ensures a feed-back control of virus replication in brain and elsewhere. Below the P^-D50 lines in each case the mice are uninfected and remain susceptible to lethal challenge. The 17D (A) strain of YFV shows a steeply rising dose for 50% infection to 10^5 p.f.u. per P^-D50 whereas the Y1 (Asibi) strain maintains a much higher efficiency of infection of about 0.04 or 25 infective units per P^-D50. For these and other YFV strains in mice of all ages the virus dose for vigorous stimulation of antibody synthesis is about x 30 that for infection and protection.

These phase-diagrams also illustrate the differentiation of virus strains by the age in mice at infection i.p. for the response to change from death to protection (diamonds in Figure 1). For an i.p. dose of 10^3 infective units this age is 3-5 days old for strain 17D (A), 13 days old for Y1 (ASIBI), 9 days old for D1 (Table 1) and 20 days old for D2 (Table 1). These distinct ages illustrate again the critical balances between pathogenic and immunogenic events that are provoked by some strains of YFV and not others. Clearly different cellular stimulations occur according to virus-strain, dose, clearance and intrinsic immunogenicity and require to be mapped as in Figure 1 before pathogenic-immunogenic interactions can be compared for different strains.

The summary Table 1 shows the essential features taken from phase-diagrams for several YFV strains. Also shown is the additional in-vivo marker for the survival time of adult mice following i.c. infection. The in-vivo 'pathogenicity' markers of the first two columns (A & B) are closely correlated inversely if the very weakly immunogenic strain 17D (A) is considered at 10^3 host-infective units (ID50) rather than 10^3 absolute-infective units (p.f.u.) as shown.

The in-vivo infection and immunogenicity markers (columns C & D) are evidently independent of the 'pathogenicity' markers and demonstrate that YFV strains may be selected to differ in at least two directions. Thus the 17D vaccine strains represent selected variants of very reduced efficiency of infection and

immunogenicity but with 'Asibi-like' activity in invasion of CNS once infection is established. By contrast, the strains D1 and D2 show unimpaired or improved efficiency of infection with enhanced (D2) or reduced (D1) vigour of CNS involvement according to the extent of immunological feed-back.

Several conclusions emerge from these results:

1) 'Virulence' is not an absolute quality of a virus strain but depends upon route of infection and dose in addition to the efficiency and quality of immune stimulation (regulatory immunization).

2) Host-susceptibility to yellow fever infection and disease develops through several stages of host-maturation in mice under 30 days old.

3) The several factors above offer a quantifiable basis (Fig. 1 & Table 1) for the in-vivo differentiation of YFV strains and for the correlation of phases of host-response with putative in-vitro, serological or genetic markers.

Legend to Figure 1

A2G or Balb C mice of the ages shown were used for the i.p. titration of infective units per LD50 (line L), infective units per 50% Antibody Conversion (line P^+) and infective units per 50% infection and protection (line P^-).

Broken lines, strain 17D (A): solid lines, strain Y1-ASIBI.

The diamonds show the ages for 50% death: protection at 10^3 i.p. infective units.

(C. J. Bradish)

Fig. 1

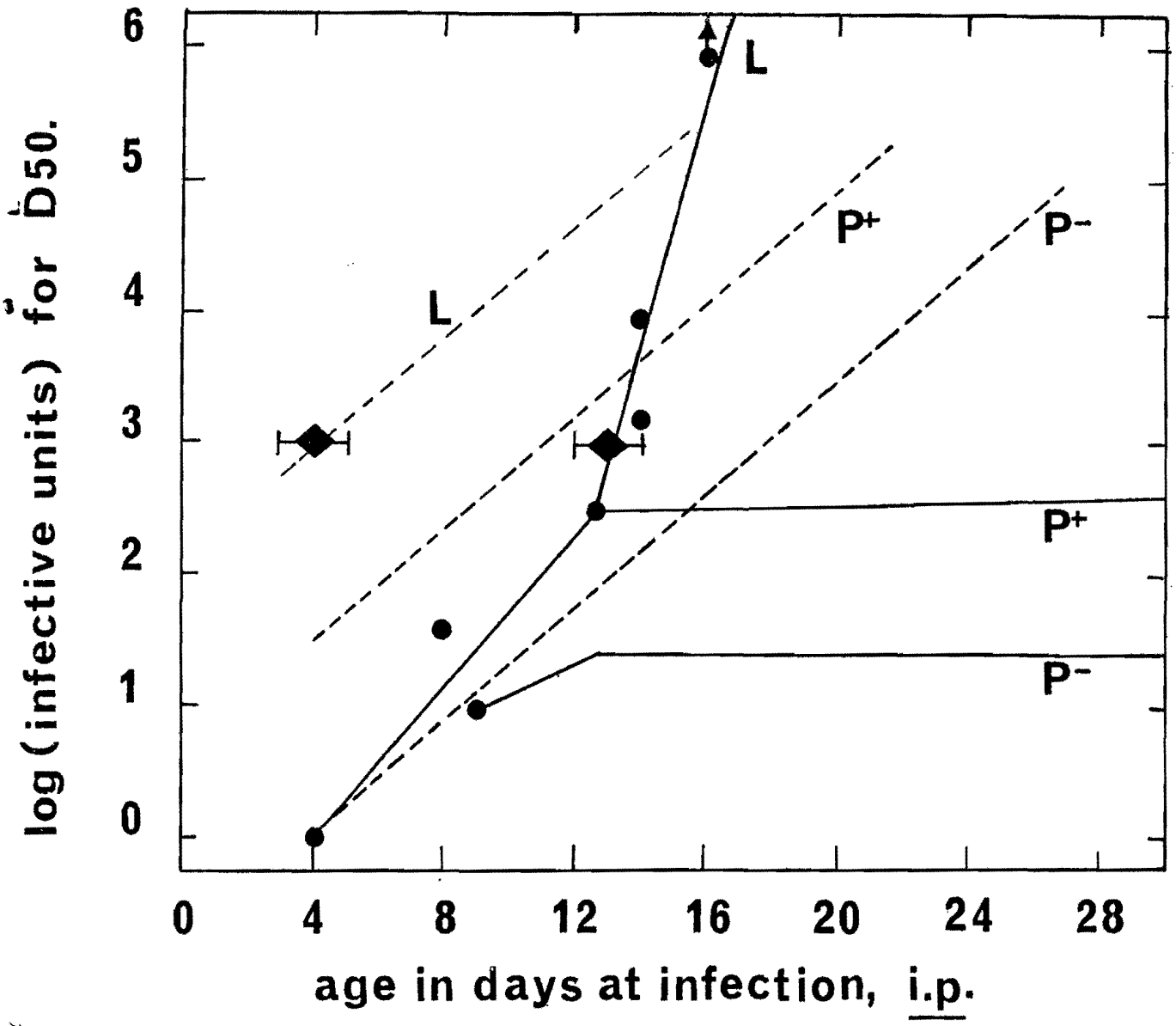
log (infective units) for D50.
6
5
4
3
2
1
0
L
L
P+
P−
P+
P−
0
4
8
12
16
20
24
28
age in days at infection, i.p.

TABLE 1: YFV STRAINS AND THE DIFFERENTIATION OF IN-VIVO QUALITIES

	A	B	C	D
STRAIN OF YFV AND PASSAGE	OUTCOME OF INFECTION BY 10^3 UNITS (VIRULENCE & REGULATORY IMMUNIZATION)		ADULT MICE, i.p., LOG INFECTIVE UNITS	
	ADULT MICE, i.c. SURVIVAL IN DAYS	AGE IN DAYS FOR 50% DEATH/PROTECTION, i.p.	PER P^-D50	PER P^+D50
D2: FRENCH NEUROTROPIC CLONE 5A: MARTIN, 1969*	5	20-21	0.5-1	1.5-2
D1: FRENCH VISCEROTROPIC CLONE 47: MARTIN, 1969*	14	8-10		
Y1: ASIBI; GHANA, 1927 HUMAN SERUM + RHESUS 53 Y3: SENEGAL, 1928 HUMAN SERUM + RHESUS 4 Y5: SENEGAL, 1953 HUMAN SERUM + MOUSE 6	8-10	10-12	1.5	2.5-3
Y25: J.S.S.; BRAZIL, 1935 HUMAN SERUM + RHESUS 3 Y27: SUAREZ; COLUMBIA, 1936 HUMAN SERUM + MOUSE 2 Y28: V141; COLUMBIA, 1959 HUMAN SERUM + MOUSE 2	8-10	10-12	2	3-4
17D (A) "ARILVAX" BURROUGHS WELLCOME LTD.: IN COMMERCIAL PRODUCTION SINCE 1938	8-10	3-5	4-5	6-7

* Annals de l'Institut Pasteur, 115, 391-403, 1969

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