Number 26 March, 1974

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

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Institute of Clinical Investigation, Faculty of Medicine, University of Zulia, Maracaibo, Venezuela
Health Institute of Colombia, Arbo-virus Section, Bogota, Colombia
Virology Department, National Institute of Hygiene "Leopoldo Izquieta Perez", Guayaquil, Ecuador
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Department of Microbiology, University of Western Australia, Perth, W.A., Australia
Department of Virology, School of Tropical Medicine, Calcutta, India

Comments from the Editor--Notice of Next Deadline

Roy W. Chamberlain, Sc.D., Editor
Mary Ellen Gist, Secretary

REVIEW OF BOOK OF GREAT INTEREST TO ARBOVIROLOGISTS

"Viruses and Invertebrates" edited by A.J. Gibbs (Research School of Biological Sciences, The Australian National University, Canberra). North-Holland Research Monographs, Frontiers of Biology, Vol. 31, N.-H. Publishing Co., Amsterdam, London. American Elsevier Publishing Co., Inc., New York. 1973 xvi + 673 pp., illus.

This is a compendium of papers contributed by 33 international workers. As pointed out by the editor, "At least three groups of virologists work with viruses and vertebrates; those who study viruses of invertebrates, and plant and mammal virologists studying vector-borne viruses. These three groups rarely meet, they work in different laboratories, publish their research and review papers in different journals and attend different meetings. This book attempts to cut across those lines."

The book is divided into five sections. The first concerns three chapters dealing with the history of the three branches of virology associated with invertebrates. Section two has a chapter describing the various viruses and 9 chapters dealing with the classification and biology of the invertebrates associated with the viruses. Section three is made up of 4 chapters of general topics of interest in virus/invertebrate studies (virus replication, infection in invertebrates, immune response in invertebrates and cell cultures). Section four (11 chapters) concerns ecology of viruses and certain invertebrates. Section five (3 chapters) deals with the control of viruses and invertebrates.

The book is well put together and is beautifully printed and illustrated.

(T.H.G. Aitken)

REPORT FROM THE CHAIRMAN OF THE SUBCOMMITTEE ON EVALUATION OF ARTHROPOD-BORNE STATUS (SEAS)

The Subcommittee on Evaluation of Arbovirus Status (SEAS) was appointed by the Chairman of the ACAV in November 1970[1] and held its first meeting in Washington 18 March 1971 with four members present. A second meeting of six members took place in December 1971 in Boston. Since then the group has continued to meet twice annually. Membership has consisted of Drs. William McD. Hammon, Chairman (recently retired), Thomas H.G. Aitken, Roy W. Chamberlain, Donald M. McLean, William C. Reeves and Albert Rudnick.

The subcommittee's mandate is as follows:

1. Formulate guidelines for deciding whether a particular virus is arthropod-borne;

2. Determine the status of each catalogued virus according to these criteria;

3. Report its decisions through the Chairman of the ACAV Executive Committee; and

4. Seek pertinent new information useful in periodic review and updating of decisions.

The subcommittee accepted the 1967 WHO definition of an arbovirus[2] as it is based on ecological interrelationships of virus, vertebrate host and arthropod vector. This definition states:

> "Arboviruses are viruses which are maintained in nature principally, or to an important extent, through biological transmission between susceptible vertebrate hosts by haematophagous arthropods; they multiply and produce viraemia in the vertebrates, multiply in the tissues of arthropods, and are passed on to new vertebrates by the bites of arthropods after a period of extrinsic incubation."

Guidelines established by the subcommittee for determining the status of a virus are as follows:

1. Isolation (once, several or many times) from a naturally infected arthropod;

2. Demonstration of biological arthropod transmission, either by:

   a. Feral naturally infected arthropods feeding on a susceptible host.

   b. Infection of a properly "isolated" sentinel animal by feeding flying arthropods.

   c. Arthropods infected by feeding on a viremic host or virus suspension.

   d. Arthropods infected after injection with a virus suspension.

3. Demonstration after a suitable incubation period, of virus in the salivary glands or entire body of arthropods infected by injection;

4. Demonstration of transtadial or transovarian infection of an arthropod vector.

5. Demonstration of viremia in:

   a. Feral vertebrates or man other than by laboratory infection.

   b. Properly "isolated" sentinel vertebrates.

   c. Laboratory-infected vertebrates, including man;

6. Antigenic relationship to a proven arbovirus;

7. Strong epidemiological associations compatible with arthropod transmission; and

8. Negative information such as:

   a. Unusual physical, chemical or biological characteristics.

   b. Failure to replicate or pass serially in a suitable arthropod.

c. Isolation from salivary glands, urine or faeces of a vertebrate host.

d. Failure to pass transtadially when isolated from ticks.

Virus information was obtained from various sources: the published catalogue of the "arboviruses" and its supplements, the working card catalogue, published papers, abstracts, laboratory annual reports, letters from directors of arbovirus laboratories and research records of subcommittee members.

On the basis of the stated guidelines, each subcommittee member evaluated a group of viruses, classifying each agent as: arbovirus, probable arbovirus, possible arbovirus, probably not arbovirus or not arbovirus. The criteria for this classification have been defined by Dr. Hammon in the October 1972 issue (No.23) of the Arthropod-borne Virus Information Exchange and will not be elaborated upon here. Suffice it to say that the determined arthropod-borne status of each virus was an almost unanimous decision of the six-member subcommittee, only a very few being relegated to a majority decision. To date, 280 viruses have been evaluated by the entire subcommittee and an additional 26 by individual members. Reevaluation of former decisions is going on currently as new information becomes available and it is hoped that by the end of the year the 309 viruses registered as of 31 December 1972 will have been classified.

A breakdown of the classification of the 280 evaluated viruses is as follows:

| | No. | % | |
|---|---|---|---|
| Arbovirus . . . . . . . . . . . . . | 82 | 29 | 45 |
| Probable arbovirus . . . . . . . . . . | 44 | 16 | |
| Possible arbovirus . . . . . . . . . . | 134 | 48 | |
| Probably not arbovirus . . . . . . . | 6 | 2 | 7 |
| Not arbovirus . . . . . . . . . . . . | 14 | 5 | |

The striking thing about this list is that only 45% of the agents are considered true or probable arboviruses whereas 48% might possibly be arthropod-borne. This situation implies ignorance--ignorance of the basic mechanisms by which these agents are maintained in nature. How can we overcome this ignorance? The subcommittee has several suggestions to make; some require additional field work while others can be pursued in the laboratory. Recommended studies (selected) useful in determining the status of a candidate arbovirus are the following:

1. Properly controlled laboratory transmission studies with the suspected vector, e.g. mosquito if this is the suspected vector or tick, etc. if it be suspect. Studying the susceptibility of a mosquito to a suspected tick-borne virus, while of interest, tells us little of what may be going on in nature.

2. Transmission studies preferably should be undertaken with feeding arthropods. However, if virus injection techniques are used, workers are urged to terminate such experiments by allowing the test arthropod to bite a susceptible host. Demonstration of virus transmission by bite is more important than recovery of virus from a triturated arthropod.

3. Greater attention should be paid to the testing of feral arthropods to see if they are naturally infected and capable of transmitting virus by bite as so ably demonstrated for jungle yellow fever by Raymond Shannon and colleagues in Brazil 35 years ago and subsequently for other viruses by workers in Brazil and Panama. This is a technique which has been long in vogue with tick workers interested in Rickettsia and relapsing fever spirochaetes. Once mechanical difficulties have been worked out, significant results may be rapidly forthcoming if the

field worker is blessed with a "hot spot" of virus activity. Care should be taken to hold field-collected arthropods sufficiently long to avoid possible mechanical transmission of virus.

4. Viremia studies in laboratory animals or wild vertebrate hosts are strongly urged. In a review of the 204 viruses published in the "Catalogue of Arthropod-borne Viruses of the World" (1967), 63 viruses were reported from human serum; there were 60 viruses from serum of lower vertebrates (natural viremia); and experimental viremias were demonstrated for 57 agents. Sad to say, there was no published evidence for viremia from among 89 viruses (44% of the total). It is possible that some of this lack of information represents careless reporting but the fact remains that important information--key information for the proper understanding of a significantly large number of viruses was missing at the time the catalogue was published.

5. Another area of experimentation woefully neglected is the determination of susceptibility of laboratory animals to subcutaneous inoculation of low passage virus. It seems strange that in a field where arthropods are the vectors of viruses, so few such studies have been undertaken.

6. Investigators, where possible, should take care to separate field-collected arthropods into pools of blooded and non-blooded specimens. Preferably, freshly-collected blooded specimens are held 24 to 48 hours to allow time for any mechanically-acquired virus or antibody to be destroyed or virus in blood to be inactivated. I want to say parenthetically that there is strong disagreement among committee members on this point. Future registrants of new viruses are asked to record such details, as the information can be important to the subcommittee in evaluating the virus.

It should be emphasized that the subcommittee can only make decisions based on available evidence. World-wide scientific efforts are essential to filling the gaps in our knowledge and are much more important than the actual deliberations of the subcommittee. Please communicate with the Chairman, Dr. Aitken, if you have useful information concerning a given virus. Please let the subcommittee know if you disagree with its judgement and why. Viruses are continually subject to review of status. Up-to-date classification is important both to students of the field and those dealing with the viruses on a day-to-day basis.

Thomas H.G. Aitken, Chairman

(The above report was presented at the Open Meeting of the American Committee on Arthropod-borne Viruses, International Congresses of Tropical Medicine and Malaria, Athens, Greece, in October, 1973, and at the annual meeting of the American Society of Tropical Medicine and Hygiene, Houston, Texas, in November, 1973.)

REFERENCES:

1. The Subcommittee on Information Exchange of the American Committee on Arthropod-borne Viruses. Catalogue of Arthropod-borne and Selected Vertebrate Viruses of the World. 1971. Am. J. Trop. Med. & Hyg., 20(6): 1018.

2. WHO Technical Report Series No. 369, 1967. Arbovirus and Human Disease. Report of a WHO Scientific Group, p.9.

## UPDATED EVALUATIONS OF ARTHROPOD-BORNE STATUS OF CATALOGUED ARBOVIRUSES

An initial evaluation of "arthropod-borne status" of all registered arboviruses was compiled by the Subcommittee on Evaluation of Arthropod-borne Status (SEAS) and published in issue No. 23 (October 1, 1972) of the Arthropod-borne Virus Information Exchange. At that time 270 agents were considered. Since then, additional information has become available and new viruses registered. Accordingly, a revised list of evaluations for 320 viruses is herewith presented (effective date November 7, 1973).

Additional information on any of the catalogued "arboviruses" which may affect judgement of their arthropod-borne status will be gratefully received. Critical information necessary for status evaluation is woefully lacking for a great many of the catalogued viruses, which relegates them to the weak "possible arbovirus" position. Thus the need for critical experiments to demonstrate the capability of suspect vector species to biologically transmit particular viruses is clearly indicated. It is hoped that recipients of the Info-Exchange will conduct such experiments and supply the critical data to the SEAS subcommittee.

Two prominent members of SEAS, Dr. W. McDowell Hammon and Dr. William C. Reeves, have left the subcommittee and two new members have been added--Dr. D. Bruce Francy of the Vector-borne Diseases Division, Center for Disease Control, Ft. Collins, Colorado, and Dr. John P. Woodall, Division of Laboratories and Research, State of New York Department of Health, Albany, New York.

Thomas H.G. Aitken, Chairman, SEAS

Roy W. Chamberlain
D. Bruce Francy
Donald M. McLean
Albert Rudnick
John P. Woodall

| Name of Virus | Abbreviation | Group Complex | Suspected Vector | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 Once only | 2 2-9 times | 3 ≥10 times | 4 By arth. infected by feeding | 5 "Isolated" sentinel animal infected | 6 By wild naturally infected arth. | 7 By arth. infected by injection | 8 Isolation from salivary glands | 9 Isolation from whole arthropod | 10 Transtadial or transovarial | 11 Experimentally infected animal or man | 12 In "isolated" sentinel (col. 5) | 13 In feral animal or man by other than lab infection | 14 Antigenically related to proven arbovirus | 15 Strong epidemiologic associations | 16 Unusual physical, chemical or biologic characteristics | 17 No replication in suspect arthropod | 18 Virus found in vertebrate salivary glands, urine, or feces | 19 Neg. transtadial passage of tick isolate | 20 Arbovirus | 21 Probable arbovirus | 22 Possible arbovirus | 23 Probably not arbovirus | 24 Not arbovirus | |
| Abal | ABAL | QRF | T | Withdrawn from catalogue (strain of Johnston Atoll, Doherty, 1973). | | | | | | | | | | | | | | | | | | | | | | | | Queensland |
| Absettarov | ABS | B | T | | | X | X? | | | | | | X | | | X | X | X | | | | | X | | | | | Leningrad USSR |
| Abu Hammad | AH | DGK | T | | X | | | | | | | | | X | | | | | | | | | | | X | | | Egypt |
| Acado | ACD | COR | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Ethiopia |
| Acara | ACA | CAP | M | | X | | | X | | | X | | | | | X | X | | | | | | | X | | | | Pará |
| African horse sickness | AHS | AHS | C | | | X | | | | | | | | X | | X | | X | | | | | X | | | | | South Africa |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| African swine fever | ASF | | T | | | X | X | | X | | | | X | X | | X | | X | X | | X | | X | | | | | Kenya |
| Aino | AINO | SIM | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Japan |
| Akabane | AKA | SIM | M+C | | X | | | | | | | X | | X | | | X | | | | | | | X | | | | Japan |
| Alfuy | ALF | B | M | X | | | X | | | | X | X | | | | X | X | | | | | | X | | | | | Queensland |
| Almpiwar | ALM | | M | | | | | | | | X | X | | | | X | | | | | | | | X | | | | Queensland |
| Amapari | AMA | TCR | | | X | | | | | | | | | X | | X | | | | | X | | | | | | X | Amapá, Brazil |
| Anhanga | ANH | PHL | | | | | | | | | | | | | | | X | | | | | | | | X | | | Pará |
| Anhembi | AMB | BUN | M | | X | | | | | | | | | | | | X | X | | | | | | | X | | | Saō Paulo |
| Anopheles A | ANA | ANA | M | ? | | ? | | | | | X | X | | X | | | | | | | | | | X | | | | Colombia |
| Anopheles B | ANB | ANB | M | | X | | | | | | | | | X | | | | | | | | | | | X | | | Colombia |
| Apeu | APEU | C | M | | X | | | X | | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Apoi | APOI | B | | | | | | | | | | | | | | X | | | | | | | | | X | | | Japan |
| Arkonam | ARK | | M | | X | | | | | | | X | | | | | | | | | | | | | X | | | S. India |
| Aruac | ARU | ARU | M | | | X | | | | | X | | | X | | | | | | | | | | X | | | | Trinidad |
| Arumowot | AMT | PHL | M | | X | | | | | | | | | | | | X | | X | | | | | | X | | | Sudan |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. 1 | 2 | 3 | Biological arth. transmission demonstrated 4 | 5 | 6 | 7 | Arth. inf. by inj. 8 | 9 | Passage 10 | Vertebrate viremia demonstrated 11 | 12 | 13 | Rel. 14 | Epi 15 | Negative information 16 | 17 | 18 | 19 | Rating 20 | 21 | 22 | 23 | 24 | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aura | AURA | A | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Pará |
| Bahig | BAH | TETE | | | | | | | | | X | | | | | X | X | | | | | | | | X | | | Egypt |
| Bakau | BAK | BAK | M+T | | | X | | | | | | | | | | X | | | | | | | | | X | | | Malaya |
| Baku | BAKU | KEM | T | | | X | | | | | | | | X | | | | | | | | | | | X | | | Caspian USSR |
| Bandia | BDA | QYB | T | | X | | | | | | | | | | | X | | | | | | | | | X | | | Senegal |
| Bangui | BGI | | | | | | | | | | | | | | | X | | | | | | | | | X | | | Central African Rep. |
| Banzi | BAN | B | M | | | X | X | X | | | | | | X | | X | X | | | | | | X | | | | | South Africa |
| Batai | BAT | BUN | M | | X | | | | | | | X | | | | | X | | | | | | | | X | | | Malaya |
| Bauline | BAU | KEM | T | X | | | | | | | | | X | | | | | | | | | | | | X | | | New Foundland |
| Bebaru | BEB | A | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Malaya |
| Belmont | BEL | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Bertioga | BER | GMA | | | | | | X | | | | | | | X | | X | | | | | | | | X | | | Saõ Paulo |
| Bhanja | BHA | | T | | | X | | | | | | | | X | | X | | | | | | | | | X | | | Orissa, India |
| Bimiti | BIM | GMA | M | | | X | | X | | | | | | X | X | X | X | X | | | | | X | | | | | Trinidad |
| Blue tongue | BLU | BLU | C | | | X | X | X | | X | | X | | X | | X | | X | X | | | | X | | | | | South Africa |

| Name of Virus | | | | Isol. from nat. infect. arth. | | Biological arth. transmission demonstrated | | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Bocas | BOC | CAL | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Panama |
| Boracea | BOR | ANB | M | | X | | | | | | | | | X | | | | | | | | | | | X | | | Saõ Paulo |
| Botambi | BOT | | M | X | | | | | | | | | | | | | X | | | | | | | | X | | | Central African Rep. |
| Boteke | BTK | BTK | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Central African Rep. |
| Bouboui | BOU | B | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Central African Rep. |
| Bujaru | BUJ | PHL | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Pará |
| Bunyamwera | BUN | BUN | M | | | X | X | | | X | X | X | | X | | X | X | X | | | | | X | | | | | Uganda |
| Burg el Arab | BEA | MTY | | | | | | | | | | | | | | X | | | | | | | | | X | | | Egypt |
| Bushbush | BSB | CAP | M | | X | | | X | X | | X | | | | | | X | | | | | | X | | | | | Trinidad |
| Bussaquara | BSQ | B | M | | | X | X | X | X | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Buttonwillow | BUT | SIM | C | | | X | X | | | | | X | | X | | X | X | X | | | | | X | | | | | California |
| Bwamba | BWA | BWA | M | | X | | | | | | X | X | | | | X | X | | | | | | | X | | | | Uganda |
| Cache Valley | CV | BUN | M | | | X | X | | | | | | | X | | | X | | | | | | X | | | | | Utah |
| California encephalitis | CE | CAL | M | | | X | X | | | X | X | X | | X | | X | X | X | | | | | X | | | | | California |
| Calovo | CVO | BUN | M | | X | | | | | ? | | X | | X | | | X | X | | | | | | | X | | | Slovakia |

| Name of Virus | a | b | c | 1 Isol. from nat. infect. arth. | 2 | 3 | 4 Biological arth. transmission demonstrated | 5 | 6 | 7 | 8 Arth. inf. by inj. | 9 | 10 Passage | 11 Vertebrate viremia demonstrated | 12 | 13 | 14 Rel. | 15 Epi | 16 Negative information | 17 | 18 | 19 | 20 Rating | 21 | 22 | 23 | 24 | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Candiru | CDU | PHL | | | | | | | | | | | | X | | X | X | | | | | | | | X | | | Pará |
| Capim | CAP | CAP | M | | | X | | X | X | | | | | | | X | X | X | | | | | X | | | | | Pará |
| Caraparu | CAR | C | M | | | X | X | X | X | | X | X | | X | X | X | X | X | | | | | X | | | | | Pará |
| Catu | CATU | GMA | M | | | X | X | X | | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Chaco | CHO | TIM | | | | | | | | | X | | | | | | | | | | | | | | X | | | Pará |
| Chagres | CHG | PHL | M | X | | | | | | | | | | | | X | X | | | | | | | | X | | | Canal Zone |
| Chandipura | CHP | VSV | P? | X | | | X | | | | | | | X | | X | X | X | | | | | | X | | | | India |
| Changuinola | CGL | CGL | P | | | X | | | | | | | | | | X | X | X | | | | | | X | | | | Panama |
| Charleville | CHV | | P | | X | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Chenuda | CNU | KEM | T | | | X | | | | | | X | | | | | | | X | | | | | | X | | | Egypt |
| Chikungunya | CHIK | A | M | | | X | X | X | | | X | X | | X | | X | X | X | | | | | X | | | | | Tanganyika |
| Chobar Gorge | CG | | T | X | | | | | | | | | | | | | | | | | | | | | X | | | Nepal |
| Cocal | COC | VSV | M? | | X | | X | X | | X | X | X | | X | | X | X | X | | | | | X | | | | | Trinidad |
| Colorado tick fever | CTF | | T | | | X | X | | | | | | X | X | | X | | X | | | | | X | | | | | Colorado |
| Congo | CON | CON | T | | | X | | | | X | | X | X | X | X | X | | | | | | | X | | | | | Zaire |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. 1 | 2 | 3 | Biological arth. transmission demonstrated 4 | 5 | 6 | 7 | Arth. inf. by inj. 8 | 9 | Passage 10 | Vertebrate viremia demonstrated 11 | 12 | 13 | Rel. 14 | Epi 15 | Negative information 16 | 17 | 18 | 19 | Rating 20 | 21 | 22 | 23 | 24 | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Corriparta | COR | COR | M | | X | | | | | | X | X | | | | | | | | | | | | | X | | | Queensland |
| Cotia (= pox virus) | COT | | M ? | | X | | | X | | | | | | X | | | | | X | X | | | | | | | X | Sao Paulo |
| Cowbone Ridge | CR | B | | | | | | | | | | | | | | | X | | | | | | | | | X | | Florida |
| D'Aguilar | DAG | PAL | C | | X | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Dakar bat | DB | B | | | | | | | | | | | | | | | X | | | | X | | | | | | X | Senegal |
| Dengue-1 | DEN-1 | B | M | | | X | X | X | | | | | | X | X | X | X | X | | | | | X | | | | | Hawaii |
| Dengue-2 | DEN-2 | B | M | | | X | X | X | | X | | X | | X | X | X | X | X | | | | | X | | | | | New Guinea |
| Dengue-3 | DEN-3 | B | M | | | X | X | | | | X | X | | | | X | X | X | | | | | X | | | | | Philippines |
| Dengue-4 | DEN-4 | B | M | | | X | | | | X | | X | | | | X | X | X | | | | | X | | | | | Philippines |
| Dera Ghazi Khan | DGK | DGK | T | X | | | | | | | | | | | | | | | | | | | | | X | | | Pakistan |
| Dhori | DHO | | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Gujorat India |
| Dugbe | DUG | GAN | T | | | X | | | | | | X | | | | X | | | | | | | | | X | | | Nigeria |
| Eastern equine encephalitis | EEE | A | M | | | X | X | X | | X | X | X | | X | X | X | X | X | | | | | X | | | | | Virginia |
| Edge Hill | EH | B | M | | | X | X | | | X | | X | | | | | X | | | | | | X | | | | | Queensland |
| Entebbe bat | ENT | B | | | | | | | | | | | | | | | X | | | | X | | | | | | X | Uganda |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Ep. Hem. Dis. | EHD | EHD | C | | | | X | | | | X | X | | X | | X | | | | | | | | X | | | | New Jersey |
| Eubenangee | EUB | EUB | M | X | | | | | | | X | X | | | | | | | | | | | | | X | | | Queensland |
| Everglades | EVE | A | M | | | X | X | | | | | | | X | | X | X | X | | | | | X | | | | | Florida |
| Flanders | FLA | | M | | | X | | | ? | | | | | | | | | | | | | | | | X | | | New York |
| Gamboa | GAM | | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Canal Zone |
| Ganjam | GAN | GAN | T+M | | | X | | | | | | X | | | | X | | | | | | | | | X | | | Orissa, India |
| Germiston | GER | BUN | M | | | X | X | X | | | | X | | X | | X | X | X | | | | | X | | | | | South Africa |
| Getah | GET | A | M | | | X | X | | | X | | X | | X | | | X | X | | | | | X | | | | | Malaya |
| Gossas | GOS | | | | | | | | | | | | | | | | | | | | X | | | | | X | | Senegal |
| Grand Arbaud | GA | UUK | T | | X | | X | | | | | | | X | | | | | | | | | X | | | | | France |
| Great Island | GI | KEM | T | | X | | | | | | | | | | | | | | | | | | | | X | | | New Foundland |
| Guajara | GJA | CAP | M | | | X | | X | X | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Guama | GMA | GMA | M | | | X | X | X | X | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Guaratuba | GTB | | M | | | X | | X | | | | | | | | X | X | X | | | | | | X | | | | Saõ Paulo |
| Guaroa | GRO | BUN | M | | | X | | | | | X | X | | X | | X | X | X | | | | | X | | | | | Colombia |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
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| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Gumbo Limbo | GL | C | M | | | X | | | | | | | | X | | X | X | X | | | | | | X | | | | Florida |
| Hanzalova (= RSSE) | HAN | B | T | | X | | | | | | | | | X | | ? | X | | | | | | X | | | | | Czechoslovakia |
| Hart Park | HP | | M | | | X | | | | | X | X | | X | | X | | | | | | | | X | | | | California |
| Hazara | HAZ | CON | T | X | | | | | | | | | | | | | | | | | | | | | X | | | Pakistan |
| Huacho | HUA | KEM | T | X | | | | | | | | | | | | | | | | | | | | | X | | | Peru |
| Hughes | HUG | HUG | T | | | X | | | | | | X | X | X | | X | | | | | | | | X | | | | Florida |
| Hypr (= RSSE) | HYPR | B | T | | | X | | | | | | | X | X | | X | X | | | | | | X | | | | | Moravia |
| Icoaraci | ICO | PHL | M,P | | | X | | X | | | | | | X | | X | X | | | | | | | X | | | | Pará |
| Ieri | IERI | | M | | X | | | | | | X | | | X | | | | | | | | | | | X | | | Trinidad |
| Ilesha | ILE | BUN | M | | X | | | | | X | | X | | X | | X | X | | | | | | | X | | | | Nigeria |
| Ilheus | ILH | B | M | | | X | X | X | | X | X | X | | X | X | X | X | X | | | | | X | | | | | Bahia, Brazil |
| Ingwavuma | ING | SIM | M | | X | | X | | | X | | X | | | | X | X | | | | | | X | | | | | South Africa |
| Inkoo | INK | CAL | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | South Finland |
| Irituia | IRI | CGL | | | | | | | | | | | | | | X | | | | | | | | | X | | | Pará |
| Israel turkey | IT | B | | | | | | | | | | X | | | | | X | | | | | | | | X | | | Israel |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. 1 | 2 | 3 | Biological arth. transmission demonstrated 4 | 5 | 6 | 7 | Arth. inf. by inj. 8 | 9 | Passage 10 | Vertebrate viremia demonstrated 11 | 12 | 13 | Rel 14 | Epi 15 | Negative information 16 | 17 | 18 | 19 | Rating 20 | 21 | 22 | 23 | 24 | Remarks |
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| Issyk-Kul | IK | | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Kirghiz |
| Itaporanga | ITP | PHL | M | | X | | | X | | | | | | X | | X | X | X | | | | | X | | | | | Saõ Paulo |
| Itaqui | ITQ | C | M | | | X | | X | | | | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Jamestown Canyon | JC | CAL | M | | | X | X | X | | | | X | | | X | | X | X | | | | | X | | | | | Colorado |
| Japanaut | JAP | | | X | | | | | | | | X | | | | X | | | | | | | | X | | | | New Guinea |
| Japanese B Encephalitis | JBE | B | M | | | X | X | X | | X | X | X | | X | X | X | X | X | | | | | X | | | | | Japan |
| Jerry Slough (JC) | JS | CAL | M | | X | | X | | | | | | | X | | | X | X | | | | | X | | | | | California |
| Johnston Atoll | JA | QRF | T | | X | | | | | | | | X | X | | | X | | | | | | | | X | | | Johnston Atoll |
| Joinjakaka | JOI | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | New Guinea |
| Juan Diaz | JD | CAP | | | | | | X | | | | | | | | | X | | | | | | | | X | | | Panama |
| Junin | JUN | TCR | | | | X | | | | | | | | X | | X | | | | | X | | | | | | X | Argentina |
| Jurona | JUR | | M | X | | | | | | | | | | | | | X | | | | | | | | X | | | Pará |
| Jutiapa | JUT | B | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Guatemala |
| Kaeng Khoi | KK | | | | | | | ? | | | | | | X | ? | | X | | | | X | | | | X | | | Thailand |
| Kairi | KRI | BUN | M | | | X | | X | | X | X | X | | X | X | | X | | | | | | X | | | | | Trinidad |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Kaisodi | KSO | KSO | T | | | X | | | | | | | | | | X | | | | | | | | | X | | | Mysore, India |
| Kamese | KAM | MOS | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Uganda |
| Kammavanpettai | KMP | | | | | | | | | | | | | X | | X | | | | | | | | | X | | | South India |
| Kannamangalam | KAN | | | | | | | | | | | | | | | X | | | | | | | | | X | | | South India |
| Kao Shuan | KS | DGK | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Taiwan |
| Karimabad | KAR | PHL | P | X | | | | | | | | | | | | | X | | | | | | | | X | | | Iran |
| Kasba | KAS | PAL | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | South India |
| Kemerovo | KEM | KEM | T | | | X | | | | | | | | X | | X | | | | | | | | X | | | | Siberia |
| Kern Canyon | KC | | | | | | | | | | | | | | | | | | X | X | X | | | | | X | | California |
| Ketapang | KET | BAK | M | X | | | | | | | X | X | | X | | | | | | | | | | X | | | | Malaya |
| Keterah | KTR | | T | | X | | | | | | X | X | | X | | X | | | | | | | | X | | | | Malaya |
| Keuraliba | KEU | | | | | | | | | | | | | | | X | | | | | | | | | X | | | Senegal |
| Keystone | KEY | CAL | M | | | X | X | X | | | | | X | X | X | X | X | X | | | | | X | | | | | Florida |
| Kokobera | KOK | B | M | | X | | | | | X | | X | | | | | X | | | | | | | X | | | | Queensland |
| Koongol | KOO | KOO | M | | X | | | | | | | X | | | | | | | | | | | | X | | | | Queensland |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Koutango | KOU | B | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Senegal |
| Kowanyama | KOW | | M | | X | | | | | | X | X | | | | | | | | | | | | | X | | | Queensland |
| Kumlinge (= CEE) | KUM | B | T | | | | | | | | | | | X | | X | X | X | | | | | X | | | | | Finland |
| Kunjin | KUN | B | M | | | X | X | | | | | X | | X | | | X | | | | | | X | | | | | Queensland |
| Kwatta | KWA | KWA | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Surinam |
| Kyasanur forest disease | KFD | B | T | | | X | X | | | | | X | X | X | | X | X | X | | | | | X | | | | | India |
| LaCrosse | LAC | CAL | M | | | X | X | X | | | | | X | X | X | X | X | X | | | | | X | | | | | Wisconsin |
| Lagos bat | LB | | | | | | | | | | | | | | | | | | | X | X | | | | | | X | Nigeria |
| La Joya | LJ | | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Panama |
| Langat | LGT | B | T | | X | | X | | | | | | X | X | | | X | | | | | | X | | | | | Malaya |
| Lanjan | LJN | KSO | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Malaya |
| Lassa | LAS | TCR | | | | | | | | | | | | | | X | | | | | X | | | | | | X | Nigeria |
| Latino | LAT | TCR | | | | | | | | | | | | | | | | | | | X | | | | | | X | Bolivia |
| Lebombo | LEB | | M | | X | | | | | | | X | | | | X | | | | | | | | X | | | | South Africa |
| Le Dantec | LD | | | | | | | | | | | | | | | X | | | | | | | | | X | | | Senegal |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Lipovnik (= Tribec) | LIK | KEM | T | | X | | | | | | | | | X | | | | | | | | | | | X | | | East Slovakia |
| Lokern | LOK | BUN | C | | | X | X | | | | | | | X | | X | X | X | | | | | X | | | | | California |
| Lone Star | LS | | T | X | | | | | | | | | | X | | | | | | | | | | | X | | | Kentucky |
| Louping Ill | LI | B | T | | | X | X | | X | | | | X | X | | X | X | X | | | | | X | | | | | Scotland |
| Lukuni | LUK | ANA | M | | X | | | | | | X | | | X | | | | | | | | | | | X | | | Trinidad |
| Machupo | MAC | TCR | | | | | | | | | | | | X | | X | | | ? | ? | X | | | | | | X | Bolivia |
| Madrid | MAD | C | M | | X | | | X | X | | | | | | | X | X | | | | | | X | | | | | Panama |
| Maguari | MAG | BUN | M | | | X | X | X | | X | X | X | | X | | X | X | X | | | | | X | | | | | Pará |
| Mahogany Hammock | MH | GMA | M | | | X | | | | | | | | | | X | X | | | | | | | | X | | | Florida |
| Main Drain | MD | BUN | C | | | X | X | | | | | | | X | | X | X | X | | | | | X | | | | | California |
| Manawa | MWA | UUK | T | | | X | | | | | | | | | | | | | | | | | | | X | | | Pakistan |
| Manzanilla | MAN | SIM | | | | | | | | | X | X | | | | X | X | | | | | | | | X | | | Trinidad |
| Mapputta | MAP | MAP | M | | X | | | | | | X | X | | | | | | | | | | | | | X | | | Queensland |
| Maprik | MPK | MAP | M | | X | | | | | X | | X | | | | | | | | | | | | X | | | | New Guinea |
| Marco | MCO | | | | | | | | | | X | | | | | | | | | | | | | | X | | | Pará |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Marituba | MTB | C | M | | X | | | X | X | | X | X | | X | X | X | X | X | | | | | X | | | | | Pará |
| Matariya | MTY | MTY | | | | | | | | | | | | | | X | | | | | | | | | X | | | Egypt |
| Matruh | MTR | TETE | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Egypt |
| Matucare | MAT | | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Bolivia |
| Mayaro | MAY | A | M | | | X | | | X | X | X | X | | X | | X | X | X | | | | | X | | | | | Trinidad |
| Melao | MEL | CAL | M | | X | | | | | | X | | | X | | | X | | | | | | | X | | | | Trinidad |
| Mermet | MER | SIM | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Illinois |
| Middelburg | MID | A | M | | | X | X | | | | | | | X | | | X | X | | | | | X | | | | | South Africa |
| Minatitlan | MNT | | | | | | | X | | | | | | | | | X | | | | | | | | X | | | Mexico |
| Minnal | MIN | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | Madras, India |
| Mirim | MIR | | M | | X | | | X | X | | X | | | | X | X | X | X | | | | | X | | | | | Pará |
| Mitchell River | MR | WAR | C | X | | | | | | | | X | | | | | | | X | | | | | | X | | | Queensland |
| Modoc | MOD | B | | | | | | | | | | | | X | | | X | | | X | X | | | | | | X | California |
| Moju | MOJU | GMA | M | | | X | | X | | | | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Mono Lake | ML | KEM | T | X | | | | | | | | | | | | | | | | | | | | | X | | | California |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
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| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Mont. Myotis leuk. | MML | B | | | | | | | | | | | | | | | X | | | | X | | | | | | X | Montana |
| Moriche | MOR | CAP | M | X | | | | | | | | | | | | | X | | | | | | | | X | | | Trinidad |
| Mossuril | MOS | MOS | M | | X | | | | | | | | | | | | | | | | | | | | X | | | Mozambique |
| Mount Elgon bat | MEB | | | | | | | | | | | X | | | | | | | | | X | | | | | X | | Kenya |
| M'Poko | MPO | TUR | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Central African Rep. |
| Mucambo | MUC | A | M | | | X | X | X | X | | X | X | | X | X | X | X | X | | | | | X | | | | | Pará |
| Murray Valley encephalitis | MVE | B | M | | X | | X | | | X | X | X | | X | | X | X | X | | | | | X | | | | | Victoria |
| Murutucu | MUR | C | M | | | X | | X | | | X | | | X | X | X | X | X | | | | | X | | | | | Pará |
| Nairobi sheep disease | NSD | | T | | | X | X | | | | | | | X | | X | | X | | | | | X | | | | | Kenya |
| Nariva (= paramyxovirus) | NAR | | | | | | | | | | | | | | | | | | | | | | | | | X | | Trinidad |
| Navarro | NAV | | | | | | | | | | X | | | | | | | | | | | | | | X | | | Colombia |
| Ndumu | NDU | A | M | | X | | | X | | | | | | X | | | X | | | | | | | X | | | | South Africa |
| Negishi | NEG | B | | | | | | | | | | | | X | | | X | | | | | | | | X | | | Japan |
| Nepuyo | NEP | C | M | | X | | | X | X | | X | | | X | | X | X | | | | | | X | | | | | Trinidad |
| Ngaingan | NGA | | C | X | | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
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| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Nkolbisson | NKO | | M | | X | | | | | | | | | | | | | | | | | | | | X | | | Cameroon |
| Nodamura (= picornavirus) | NOD | | | X | | | ? | | | | | X | | X | | | | | X | | | | | | | X | | Japan |
| Northway | NOR | BUN | M | | X | | | X | | | | | | | X | | X | | | | | | | X | | | | Alaska |
| Ntaya | NTA | B | M | | X | | | | | X | X | X | | X | | | X | | | | | | | X | | | | Uganda |
| Nyamanini | NYM | | T | | | X | | | | | | | | X | | X | | X | | | | | | X | | | | South Africa |
| Nyando | NDO | NDO | M | | X | | | | | | | X | | X | | X | | | | | | | | X | | | | Kenya |
| Okhotskiy | OKH | KEM | T | | X | | | | | | | | | | | | | | | | | | | | X | | | E. USSR |
| Okola | OKO | | M | X | | | | | | | | | | | | | | | | | | | | | X | | | Cameroon |
| Omsk hem. fever | OMSK | B | T | | | X | X | | | | | | X | X | | X | X | X | | | | | X | | | | | W. Siberia |
| O'nyong Nyong | ONN | A | M | | | X | X | | | | X | X | | X | X | X | X | X | | | | | X | | | | | Uganda |
| Oriboca | ORI | C | M | | | X | X | X | X | | X | X | | X | X | X | X | X | | | | | X | | | | | Pará |
| Oropouche | ORO | SIM | M | | X | | | | | | X | X | | X | | X | X | X | | | | | | X | | | | Trinidad |
| Ossa | OSSA | C | M | | X | | | | X | | | | | | | X | X | | | | | | X | | | | | Panama |
| Pacora | PCA | | M | | X | | | | | | | | | | | | | | | | | | | | X | | | Panama |
| Pacui | PAC | PHL | P | | | X | | X | | | | | | X | | X | X | | | | | | | X | | | | Pará |

| | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Name of Virus | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | Remarks |
| Pahayokee | PAH | PAT | M | | | X | | | | | | | | X | | | X | | | | | | | | X | | | Florida |
| Palyam | PAL | PAL | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | South India |
| Parana | PAR | TCR | | | | | | | | | | | | | | | | | | | | | | | | | X | Paraguay |
| Pathum Thani | PTH | DGK | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Thailand |
| Patois | PAT | PAT | M | | X | | | X | X | | | | | | | X | X | | | | | | X | | | | | Panama |
| Phnom-Penh bat | PPB | B | | | | | | | | | | | | ? | | | X | | | | X | | | | | X | | ex-bat W. Africa |
| Pichinde | PIC | TCR | | | | X | | | | | | | | | | X | | | X | | | | | | | | X | Colombia |
| Piry | PIRY | VSV | | | | | | | | | X | | | X | | | X | | | | | | | | X | | | Pará |
| Pixuna | PIX | A | | | X | | | | | | | | | | | | X | | | | | | | | X | | | Pará |
| Pongola | PGA | BWA | M | | | X | X | | | | | | | X | | | X | | | | | | X | | | | | South Africa |
| Ponteves | PTV | UUK | T | | X | | | | | | | | X | | | | | | | | | | | | X | | | France |
| Powassan | POW | B | T | | | X | X | | | | | | X | X | | X | X | X | | | | | X | | | | | Ontario |
| Punta Salinas | PS | HUG | T | X | | | | | | | | | | | | | | | | | | | | | X | | | Peru |
| Punta Toro | PT | PHL | P | | | X | | | | | | | | | | X | X | | | | | | | | X | | | Canal Zone |
| Qalyub | QYB | QYB | T | | X | | | | | | | X | | | | | | | | | | | | | X | | | Egypt |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Quarafil | QRF | QRF | T | | | X | X | | | | | | X | X | | X | | X | | | | | X | | | | | Egypt |
| Restan | RES | C | M | | X | | X | | | | | | | X | | X | X | | | | | | X | | | | | Trinidad |
| Rift Valley fever | RVF | | M | | | X | X | | | | | | | X | | X | | X | | | | | X | | | | | Kenya |
| Rio Bravo | RB | B | | | | | | | | | | | | X | | | X | | | | X | X | | | | | X | California |
| Ross River | RR | A | M | | X | | X | | | X | | X | | X | | X | X | X | | | | | X | | | | | Queensland |
| Royal Farm | RF | B | T | | X | | | | | | | | | | | | X | | | | | | | | X | | | Afganistan |
| Russian spring summer encephalitis | RSSE | B | T | | | X | X | | | | | | X | X | | X | X | X | | | | | X | | | | | E. Siberia |
| Sabo | SABO | SIM | C | | X | | | | | | | | | | | X | X | | | | | | | | X | | | Nigeria |
| Saboya | SAB | B | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Senegal |
| Sagiyama | SAG | A | M | | | X | | X | | | | | | X | | | X | X | | | | | | X | | | | Japan |
| Sakhalin | SAK | | T | | | X | | | | | | X | X | | | | | | | | | | | | X | | | E. USSR |
| Salehabad | SAL | PHL | P | X | | | | | | | | | | | | | X | | | | | | | | X | | | Iran |
| Samford | SAM | SIM | C | | X | | | | | | | X | | | | | X | | | | | | | | X | | | Queensland |
| San Angelo | SA | CAL | M | | X | | | | | | | | | | | | X | X | | | | | | | X | | | Texas |
| Sandfly F (Naples) | SFN | PHL | P | | X | | X | | | | | | | | | X | X | X | | | | | X | | | | | Italy |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Sandfly F (Sicilian) | SFS | PHL | P | | | X | X | | | | | | X | | | X | X | X | | | | | X | | | | | Sicily |
| Sango | SAN | SIM | C | | X | | | | | | | | | | | X | X | | | | | | | | X | | | Nigeria |
| Sathuperi | SAT | SIM | M | | X | | | | | | | | | | | X | X | | | | | | | | X | | | Madras, India |
| Sawgrass | SAW | | T | | | X | | | | | | | | | | | | | | | | | | | X | | | Florida |
| Sembalan | SEM | | | | | | | | | | | | | | | X | | | | | | | | | X | | | South India |
| Semliki forest | SF | A | M | | X | | X | X | | X | X | X | | X | | | X | | | | | | X | | | | | Uganda |
| Sepik | SEP | B | M | | X | | | | | | | X | | X | | | X | | | | | | | X | | | | New Guinea |
| Shamonda | SHA | SIM | C | | X | | | | | | | | | | | X | X | | | | | | | | X | | | Nigeria |
| Shark River | SR | PAT | M | | | X | | X | | | | | | X | | X | X | X | | | | | | X | | | | Florida |
| Shuni | SHU | SIM | C | | X | | | | | | | | | | | X | X | ? | | | | | | | X | | | Nigeria |
| Silverwater | SIL | KSO | T | | | X | | | | | | | X | X | | X | | X | | | | | | X | | | | Ontario |
| Simbu | SIM | SIM | M | | X | | | | | | X | X | | X | | | X | | | | | | | X | | | | South Africa |
| Simian hem. fever | SHF | | | | | | | | | | | | | X | | ? | | | | | | | | | | | X | ? India |
| Sindbis | SIN | A | M | | | X | X | X | | X | | X | | X | | X | X | X | | | | | X | | | | | Egypt |
| Sixgun City | SC | KEM | T | | | X | | | | | | | | | | | | | | | | | | | X | | | Texas |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| St. Louis encephalitis | SLE | B | M | | | X | X | X | X | X | X | X | | X | X | X | X | X | | | | | X | | | | | Missouri |
| Sokuluk | SOK | B | | | | | | | | | | | | | | X | X | | | | | | | | X | | | Kirghiz |
| Soldado | SOL | HUG | T | | X | | | | | | | | | | | | | | | | | | | | X | | | Trinidad |
| Sororoca | SOR | BUN | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Pará |
| Spondweni | SPO | B | M | | | X | X | | | | | | | X | | X | X | | | | | | X | | | | | South Africa |
| Stratford | STR | B | M | | X | | | | | | | X | | | | | X | | | | | | | | X | | | Queensland |
| Tacaiuma | TCM | ANA | M | | X | | | X | | | X | | | X | X | ? | | | | | | | | X | | | | Pará |
| Tacaribe | TCR | TCR | | ? | | | | | | | | | | X | | | | | | X | X | | | | | | X | Trinidad |
| Tahyna | TAH | CAL | M | | | X | X | X | | X | X | X | | X | X | X | X | | | | | | X | | | | | Slovakia |
| Tamiami | TAM | TCR | | | | | | | | | | | | X | | X | | | | | X | | | | | | X | Florida |
| Tanga | TAN | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | Tanzania |
| Tataguine | TAT | | M | | X | | | | | | | | | | | X | | X | | | | | | | X | | | Senegal |
| Tembe | TME | | M | | X | | | | | | | | | | | | | | | | | | | | X | | | Pará |
| Tembusu | TMU | B | M | | | X | | X | | | | | | | | | X | X | | | | | | X | | | | Malaya |
| Tensaw | TEN | BUN | M | | | X | X | X | | | | | | X | X | X | X | X | | | | | X | | | | | Alabama |

| Name of Virus | | | | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | a | b | c | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Tete | TETE | TETE | | | | | | | | | | X | | | | | X | | | | | | | | X | | | South Africa |
| Thimiri | THI | SIM | | | | | | | | | | | | | | X | X | | | | | | | | X | | | South India |
| Thogoto | THO | THO | T | | | X | | | | | | | | | | X | | X | | | | | | | X | | | Kenya |
| Thattapalayam | TPM | | | | | | | | | | | | | | | | | | | | | | | | X | | | South India |
| Timbo | TIM | TIM | | | | | | | | | | | | | | | | | | | | | | | X | | | Pará |
| Tlacotalpan | TLA | BUN | M | | X | | | | | | | | | | | | X | | | | | | | | X | | | Mexico |
| Toure | TOU | | | | | | | | | | | | | | | | | | | | | | | | X | | | Senegal |
| Tribec | TRB | KEM | T | | X | | | X | | | | X | | X | X | X | | | X | | | | | X | | | | S.W. Slovakia |
| Triniti | TNT | | M | X | | | | | | X | X | X | | X | | | | | | | | | | X | | | | Trinidad |
| Trivittatus | TVT | CAL | M | | | X | | X | | | | | | X | X | X | X | X | | | | | | X | | | | N. Dakota |
| Trubanaman | TRU | MAP | M | X | | | | | | X | | X | | | | | | | | | | | | | X | | | Queensland |
| Tsuruse | TSU | TETE | | | | | | | | | | X | | | | X | X | | | | | | | | X | | | Japan |
| Turlock | TUR | TUR | M | | | X | X | X | X | | X | X | | X | X | X | | X | | | | | X | | | | | California |
| Tyuleniy | TYU | B | T | | X | | X | | | | | | X | | | | X | | | | | | | X | | | | E. USSR |
| Uganda S | UGS | B | M | X | | | X | X | | X | X | X | | X | | | X | X | | | | | X | | | | | Uganda |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Umatilla | UMA | | M | | | X | X | | | | | | | X | | X | | | | | | | X | | | | | Oregon |
| Umbre | UMB | TUR | M | | | X | | | | | X | | | | | X | X | | | | | | | X | | | | Bombay, India |
| Una | UNA | A | M | | | X | | X | | | | | | X | | | X | | | | | | | X | | | | Para' |
| Upolu | UPO | | T | | X | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Usutu | USU | B | M | | X | | | | | | | | | | | X | X | | | | | | | | X | | | South Africa |
| Uukuniemi | UUK | UUK | T | | | X | X | | | | | | X | X | | X | X | X | | | | | | X | | | | Finland |
| Vellore | VEL | PAL | M | X | | | | | | | | | | | | | | | | | | | | | X | | | South India |
| Venezuelan equine encephalitis | VEE | A | M | | | X | X | X | X | | | X | | X | X | X | X | X | | | | | X | | | | | Venezuela |
| Venkatapuram | VKT | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | Madras, India |
| VSV-Indiana | VSI | VSV | P? | | X | | X | X | | X | X | X | X | X | | X | X | X | | | | | X | | | | | Indiana |
| VSV-New Jersey | VSNJ | VSV | | X | | | | | | | X | X | | | | | X | | | | X | | | | X | | | New Jersey |
| Wad Medani | WM | KEM | T | | X | | | | | | | X | X | | | | | | | | | | | | X | | | Sudan |
| Wallal | WAL | | M | | X | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Wanowrie | WAN | | T | | X | | | | | | | | | X | | | | | | | | | | | X | | | Poona, India |
| Warrego | WAR | WAR | C | | X | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |

| Name of Virus | a | b | c | Isol. from nat. infect. arth. | | | Biological arth. transmission demonstrated | | | | Arth. inf. by inj. | | Passage | Vertebrate viremia demonstrated | | | Rel. | Epi | Negative information | | | | Rating | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | |
| Wesselsbron | WSL | B | M | | | X | X | | | | X | | | X | | X | X | X | | | | | X | | | | | S. Africa |
| Western equine enceph. | WEE | A | M | | | X | X | X | X | X | X | X | | X | X | X | X | X | | | | | X | | | | | California |
| West Nile | WN | B | M | | | X | X | X | | X | X | X | X | X | | X | X | X | | | | | X | | | | | Uganda |
| Whataroa | WHA | A | M | | X | | | | | X | X | X | | X | | | X | X | | | | | X | | | | | New Zealand |
| Witwatersrand | WIT | | M | | | X | | X | | | X | X | | X | | X | | | | | | | | X | | | | South Africa |
| Wongal | WON | KOO | M | | X | | | | | | | X | | | | | X | | | | | | | X | | | | Queensland |
| Wongorr | WGR | | M | X | | | | | | | | X | | | | | | | | | | | | | X | | | Queensland |
| Wyeomyia | WYO | BUN | M | | | X | | | | | X | | | X | | X | X | | | | | | | X | | | | Colombia |
| Yaquina Head | YH | KEM | T | | | X | | | | | | | | | | | | | | | | | | | X | | | Oregon |
| Yellow fever | YF | B | M | | | X | X | X | | X | X | X | | X | X | X | X | X | | | | | X | | | | | Ghana |
| Yogue | YOG | | | | | | | | | | | | | | | | | | | | | | | | X | | | Senegal |
| Zaliv Terpeniya | ZT | | | | X | | | | | | | | | | | | X | | | | | | | | X | | | E. USSR |
| Zegla | ZEG | PAT | | | | | | X | | | | | | | | X | X | | | | | | | | X | | | Panama |
| Zika | ZIKA | B | M | | | X | X | X | | | X | X | | X | X | X | X | X | | | | | X | | | | | Uganda |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

# LISTING OF ARBOVIRAL REAGENTS AVAILABLE FROM THE RESEARCH RESOURCES BRANCH, NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND

The following tables list the arbovirus seeds, immune ascitic fluids, inactivated antigens and immune grouping fluids currently available (as of March, 1974) from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014 (Telephone: 301-496-5937).

Requests for the reagents should be submitted on form NIH-381-2, "Requisition for Research Materials" and forwarded to the above address. Inquiries for further information concerning the reagents should be directed to the attention of the Chief, Research Resources Branch, Building 31, Room 7A-11.

Some of the seed virus materials require United States Public Health Service and/or United States Department of Agriculture permits before they can be distributed. Please see the numerical notation beside each seed virus listing for permit requirement: 0, (no permit required); 1, (USDA permit required); 2, (PHS permit required); 3, (both USDA and PHS permits required). USDA permits are obtained from the Chief Staff Veterinarian, Organisms and Vectors, APHIS, USDA, Hyattsville, Maryland 20782. PHS permits are obtained from the Office of Biohazards, Center for Disease Control, Atlanta, Georgia 30333.

| Catalog Number | Arbovirus Type | Strain | Volume |
|---|---|---|---|
| V517-001-522 | Anopheles A-seed virus(3) | ATCC VR 327 | 0.5 ml. |
| V517-701-562 | Anopheles A-IAF* | ATCC VR 327 | 0.5, 1.0 ml. |
| V546-001-522 | Anopheles B-seed virus(3) | ATCC VR 86 | 0.5 ml. |
| V546-701-562 | Anopheles B-IAF | ATCC VR 86 | 1.0 ml. |
| V569-001-522 | Bebaru-seed virus(2) | ATCC VR 600 | 0.5 ml. |
| V527-002-522 | Bimiti-seed virus(3) | ATCC VR 405 | 0.5 ml. |
| V519-001-522 | Bluetongue-seed virus(1) | 8 | 0.5 ml. |
| V565-001-522 | Bunyamwera-seed virus(3) | ATCC VR 87 | 0.5 ml. |
| V561-001-522 | Bussuquara-seed virus(3) | ATCC VR 557 | 0.5 ml. |
| V534-001-522 | Buttonwillow-seed virus(1) | MP 7956 | 0.5 ml. |
| V534-701-562 | Buttonwillow-IAF | MP 7956 | 0.5, 2.0, 5.0 m |
| V501-001-522 | Bwamba-seed virus(3) | Smithburn M459 | 0.5 ml. |
| V501-701-562 | Bwamba-IAF | Smithburn M459 | 0.5, 2.0 ml. |
| V502-001-522 | Calif. Encep.-seed virus(1) | BFS 283 | 0.5 ml. |
| V502-701-562 | Calif. Encep.-IAF | BFS 283 | 0.5, 2.0 ml. |
| V555-001-522 | Candiru-seed virus(3) | ATCC VR 408 | 0.5 ml. |
| V555-701-562 | Candiru-IAF | ATCC VR 408 | 1.0 ml. |
| V543-001-522 | Caraparu-seed virus(3) | ATCC VR 307 | 0.5 ml. |
| V528-001-522 | Catu-seed virus(3) | TRVL 32046 | 0.5 ml. |
| V523-001-522 | Chagres-seed virus(3) | JW-10 | 0.5 ml. |
| V523-701-562 | Chagres-IAF | JW-10 | 1.0 ml. |
| V529-001-522 | Changuinola-seed virus(3) | BT 436 | 0.5 ml. |
| V529-701-562 | Changuinola-IAF | BT 436 | 1.0 ml. |
| V548-001-522 | Chikungunya-seed virus(3) | ATCC VR 64 | 0.5 ml. |
| V548-701-562 | Chikungunya-IAF | ATCC VR 64 | 1.0 ml. |
| V514-001-522 | Cocal-seed virus(3) | ATCC VR 435 | 0.5 ml. |
| V506-001-522 | Col. Tick Fev.-seed virus(1) | Florio | 0.5 ml. |
| V506-701-562 | Col. Tick Fev.-IAF | Florio | 0.5, 2.0, 5.0 m |

| Catalog Number | Arbovirus Type | Strain | Volume |
|---|---|---|---|
| V533-001-522 | Cowbone Ridge-seed Virus(0) | NCDC | 0.5 ml. |
| V515-001-522 | EEE-seed Virus (3) | Massachusetts | 0.5 ml. |
| V515-701-562 | EEE-IAF | Massachusetts | 1.0, 2.0, 5 |
| V535-001-522 | EHD-seed virus (1) | New Jersey | 0.5 ml. |
| V568-001-522 | Getah-seed virus (3) | ATCC VR 369 | 0.5 ml. |
| V510-001-522 | Guama-seed virus (3) | TRVL 33579 | 0.5 ml. |
| V510-701-562 | Guama-IAF | TRVL 33579 | 0.5, 2.0, 5 |
| V531-001-522 | Guaroa-seed virus (3) | ATCC VR 394 | 0.5 ml. |
| V531-701-562 | Guaroa-IAF | ATCC VR 394 | 0.5, 1.0 ml |
| V516-001-522 | Gumbo Limbo-seed virus (0) | ATCC VR 619 | 0.5 ml. |
| V539-001-522 | Hart Park-seed virus (1) | ATCC VR 441 | 0.5 ml. |
| V539-701-562 | Hart Park-IAF | ATCC VR 441 | 0.5, 1.0 ml |
| V513-001-522 | Hughes-seed virus (1) | DT-1 | 0.5 ml. |
| V513-701-562 | Hughes-IAF | DT-1 | 0.5, 1.0 ml |
| V509-001-522 | Ilheus-seed virus (3) | TRVL 5800 | 0.5 ml. |
| V509-701-562 | Ilheus-IAF | TRVL 5800 | 0.5, 2.0, 5 |
| V557-001-522 | Junin-seed virus (3) | ATCC VR 525 | 0.5 ml. |
| V552-001-522 | Kern Canyon-seed virus (0) | ATCC VR 551 | 0.5 ml. |
| V552-701-562 | Kern Canyon-IAF | ATCC VR 551 | 1.0, 2.0, 5 |
| V551-001-522 | Klamath (Microtus)-seed virus (0) | 1056 | 0.5 ml. |
| V570-001-522 | Lokern-seed virus (0) | ATCC VR 620 | 0.5 ml. |
| V571-001-522 | Lone Star-seed virus (0) | ATCC VR 621 | 0.5 ml. |
| V540-001-522 | Manzanilla-seed virus (3) | ATCC VR 428 | 0.5 ml. |
| V540-701-562 | Manzanilla-IAF | ATCC VR 428 | 0.5, 1.0 ml |
| V507-001-522 | Mayaro-seed virus (3) | TRVL 15537 | 0.5 ml. |
| V507-701-562 | Mayaro-IAF | TRVL 15537 | 0.5, 2.0, 5 |
| V511-701-562 | Melao-IAF | TRVL 9375 | 0.5 ml. |
| V541-001-522 | MML-seed virus (1) | ATCC VR 537 | 0.5 ml. |
| V541-701-562 | MML-IAF | ATCC VR 537 | 0.5, 1.0 ml |

| r | Arbovirus Type | Strain | Volume |
|---|---|---|---|
| | Modoc-seed virus(1) | ATCC VR 415 | 0.5 ml. |
| | Modoc-IAF | ATCC VR 415 | 0.5 ml, 1.0 |
| | Nepuyo-seed virus(3) | TRVL 18462 | 0.5 ml. |
| | Nepuyo-IAF | TRVL 18462 | 1.0 ml. |
| | Oriboca-seed virus(3) | TRVL 47827 | 0.5 ml. |
| | Oropouche-seed virus(3) | TR 9760 | 0.5 ml. |
| | Oropouche-IAF | TR 9760 | 0.5, 2.0 ml |
| | Patois-seed virus(3) | ATCC VR 549 | 0.5 ml. |
| | Patois-IAF | ATCC VR 549 | 1.0, 2.0, 5 |
| | Phleb.(SF) Fever-seed virus(3) | Sicilian | 0.5 ml. |
| | Phleb.(SF) Fever-IAF | Sicilian | 0.5, 2.0, 5 |
| | Phleb.(SF) Fever-seed virus(3) | Naples | 0.5 ml. |
| | Phleb.(SF) Fever-IAF | Naples | 0.5, 2.0, 5 |
| | Pixuna-seed virus(3) | ATCC VR 372 | 0.5 ml. |
| | Powassan-seed virus(3) | Byers | 0.5 ml. |
| | Punta Toro-seed virus(2) | ATCC VR 559 | 0.5 ml. |
| | Punta Toro-IAF | ATCC VR 559 | 1.0 ml. |
| | Rio Bravo-seed virus(1) | M64 | 0.5 ml. |
| | Rio Bravo-IAF | M64 | 1.0 ml. |
| | Ross River-seed virus(3) | ATCC VR 373 | 0.5 ml. |
| | Ross River-IAF | ATCC VR 373 | 1.0 ml. |
| | Sawgrass-seed virus(0) | B64A-1247 | 0.5 ml. |
| | Semliki Forest-seed virus(3) | ATCC VR 67 | 0.5 ml. |
| | Silverwater-seed virus(3) | #131 | 0.5 ml. |
| | Silverwater-IAF | #131 | 1.0, 2.0, 5 |
| | Sindbis-seed virus(3) | ATCC VR 68 | 0.5 ml. |
| | SLE-seed virus(0) | Parton | 0.5 ml. |
| | Tacaribe-seed virus(3) | TRVL 11537 | 0.5 ml. |
| | Tembusu-seed virus(3) | ATCC VR 381 | 0.5 ml. |
| | Tensaw-seed virus(0) | A9-171B | 0.5 ml. |
| | Trivittatus-seed virus(0) | ATCC VR 402 | 0.5 ml. |

| Catalog Number | Arbovirus Type |
|---|---|
| V504-002-522 | Turlock-seed virus(0) |
| V504-702-562 | Turlock-IAF |
| V532-001-522 | VEE-seed virus(1) |
| V532-701-562 | VEE-IAF |
| V520-001-522 | VSV-seed virus(1) |
| V520-701-562 | VSV-IAF |
| V520-011-522 | VSV-seed virus(1) |
| V520-711-562 | VSV-IAF |
| V521-001-522 | WEE-seed virus(0) |
| V521-011-522 | WEE-seed virus(0) |
| V554-001-522 | West Nile-seed virus(3) |
| V554-701-562 | West Nile-IAF |
| V508-001-522 | Wyeomyia-seed virus(3) |
| V508-711-562 | Wyeomyia-IAF |
| V525-001-522 | Yellow Fever-seed virus(3) |
| V525-701-562 | Yellow Fever-IAF |
| V556-001-522 | Zegla-seed virus(2) |
| V556-701-562 | Zegla-IAF |

* IAF - immune ascitic fluid
0 - No permit required
1 - USDA permit required
2 - PHS permit required
3 - Both USDA and PHS permits required

| Strain | Volume |
| --- | --- |
| ATCC VR 328 | 0.5 ml. |
| ATCC VR 328 | 0.5, 1.0 ml. |
| TC 83 | 0.5 ml. |
| TC 83 | 1.0 ml. |
| Indiana | 0.5 ml. |
| Indiana | 0.5, 2.0, 5.0 ml. |
| New Jersey | 0.5 ml. |
| New Jersey | 1.0, 2.0, 5.0 ml. |
| Fleming | 0.5 ml. |
| Highlands J | 0.5 ml. |
| ATCC VR 82 | 0.5 ml. |
| ATCC VR 82 | 1.0 ml. |
| TRVL 8349 | 0.5 ml. |
| ATCC VR 91 | 1.0, 2.0, 5.0 ml. |
| 17D | 0.5 ml. |
| 17D | 1.0, 2.0, 5.0 ml. |
| ATCC VR 556 | 0.5 ml. |
| ATCC VR 556 | 1.0 ml. |

BPL INACTIVATED CF ANTIGENS

| Catalog Number | Arbovirus Type | Volume |
| --- | --- | --- |
| G210-101-522 | Lanjan | 0.5 ml. |
| G209-101-522 | Semliki Forest | 0.5 ml. |
| G215-101-522 | Capim | 0.5 ml. |
| G203-101-522 | Mermet | 0.5 ml. |
| G201-101-549 | Caraparu | 0.5 ml. |
| G207-101-549 | Tacaribe | 0.5 ml. |
| G216-101-522 | Yellow Fever | 0.5 ml. |
| G205-101-522 | Tensaw | 0.5 ml. |
| G202-101-522 | Bimiti | 0.5 ml. |
| G204-101-522 | Cocal | 0.5 ml. |

ARBOVIRUS GROUPING FLUIDS CURRENTLY AVAILABLE FOR DISTRIBUTION

| | | | Antigens Used in Preparation o Grouping Fluids | |
|---|---|---|---|---|
| RRB Catalog # | Volume | Type | Viruses | Strains |
| G201-701-567 | 1.0, 2.0 ml. | Group C | Marituba | Be An 15 |
| | | | Apeu | Be An 848 |
| | | | Madrid | Prototype |
| | | | Ossa | Prototype |
| | | | Nepuyo | Be An 107 |
| | | | Caraparu | Be An 399 |
| | | | Murutucu | Be An 974 |
| | | | Itaqui | Be An 127 |
| | | | Oriboca | Be An 17 |
| G202-701-567 | 1.0 ml. | Group Guama | Guama | Be An 277 |
| | | | Catu | Be H 151 |
| | | | Moju | Be Ar 125 |
| | | | Bimiti | TRVL 8362 |
| G203-701-567 | 1.0, 2.0 ml. | Group Simbu | Simbu | SA AR 53 |
| | | | Ingwavuma | SA An 416 |
| | | | Akabane | Ja G Ar 3 |
| | | | Manzanilla | TRVL 3587 |
| | | | Oropouche | TRVL 9760 |
| | | | Utinga | Be An 847 |
| | | | Sathuperi | IG 11155 |
| | | | Yaba-7 | -- |
| | | | Buttonwillow | A 7956 |
| G204-701-567 | 1.0, 2.0 ml. | Group VSV | VSV-Indiana | Indiana L |
| | | | VSV-New Jersey | Hazelhurs |
| | | | Cocal | TRVL 4023 |
| G205-701-567 | 1.0, 2.0 ml. | Group Bunyamwera | Bunyamwera | Original |
| | | | Germiston | SA AR 105 |
| | | | Kairi | Be AR 822 |
| | | | Taiassui | Be AR 671 |
| | | | Ilesha | Ko/2 |
| | | | Cache Valley | Holden |
| | | | Tensaw | A9 171b |
| | | | Sororoca | Be AR 321 |
| | | | Batai | MM 2222 |
| | | | Guaroa | Co H 3521 |
| | | | Maguari | Be Ar 727 |
| | | | Wyeomyia | Original |

| RRB Catalog # | Volume | Type | Antigens Used in Preparation of Grouping Fluids: Viruses | Strains |
|---|---|---|---|---|
| G206-701-567 | 1.0, 2.0 ml. | Group California | California | BFS 283 |
| | | | Trivittatus | Original |
| | | | Melao | TR 9375 |
| | | | Be AR 103645 | Be AR 103645 |
| | | | Tahyna | 92 |
| G207-701-567 | 1.0, 2.0 ml. | Group Tacaribe | Tacaribe | TRVL 11573 |
| | | | Amapari | Be An 70563 |
| | | | Junin | XJ |
| | | | Pichinde | Co An 3739 |
| | | | Tamiami | W 10777 |
| G208-701-567 | 1.0, 2.0 ml. | Group Phlebotomus | Anhanga | Be An 46852 |
| | | | Be An 100049 | Be An 100049 |
| | | | Bujaru | Be An 47693 |
| | | | Candiru | Be H 22511 |
| | | | Chagres | JW 10 |
| | | | Co Ar 3319 | Co Ar 3319 |
| | | | Eg An 754-61 | Eg An 754-61 |
| | | | Karimibad | I 58 |
| | | | Icoaraci | Be An 24262 |
| | | | Itaporanga | Prototype |
| | | | Naples | Sabin |
| | | | Punta Toro | Balliet |
| | | | Sicilian | Sabin |
| | | | Arumowot | Ar 1284-64 |
| G209-701-567 | 1.0, 2.0 ml. | Group A | EEE | Ten Broeck |
| | | | WEE | McMillan |
| | | | VEE | Trinidad Donkey TC80 |
| | | | Pixuna | BE AR 35645 |
| | | | Una | BE AR 13136 |
| | | | Mucambo | BE An 8 |
| | | | Y-62-33 | -- |
| | | | Semliki | Original |
| | | | O'nyong-nyong | MP 30 |
| | | | Chikungunya | Ross |
| | | | Ross River | T 48 |
| | | | Getah | MM 2021 |
| | | | Bebaru | MM 2354 |
| | | | Sindbis | MM 2215 |
| | | | Mayaro | TR 4675 |
| | | | Middelburg | SA Ar 749 |
| | | | Nduma | SA Ar 2211 |
| | | | Aura | BE Ar 10315 |

ARBOVIRUS GROUPING FLUIDS (Continued)

| RRB Catalog # | Volume | Type | Antigens Used in Preparation of Grouping Fluids — Viruses | Strains |
|---|---|---|---|---|
| G210-601-567 | 1.0, 2.0 ml. | Polyvalent Quaranfil | Quaranfil | Ar 1113 |
| | | | Kaisodi | IG 14132 |
| | | | Bandia | IPD/A 611 |
| | | | Johnston Atoll | Prototype |
| | | | Qalyub | EgAr 370 |
| | | | Silverwater | 131 |
| | | | Lanjan | TP 94 |
| G211-601-567 | 1.0, 2.0 ml. | Poly. Anopheles A | Anopheles A | Roca-Carcia |
| | | | Lukuni | TR 10076 |
| | | | Anopheles B | Roca-Carcia |
| | | | Boracea | SPAr 395 |
| | | | Tacaiuma | Be An 73 |
| | | | CoAr 1071 | CoAr 1071 |
| | | | CoAr 3624 | CoAr 3624 |
| | | | Turlock | S-1954-847-32 |
| | | | Umbre | IG 1424 |
| | | | Yaba-1 | Y-1 |
| G212-601-567 | 1.0, 2.0 ml. | Polyvalent Bwamba | Bwamba | M459 |
| | | | Pongola | SA Tar 1 |
| | | | Mossuril | Ar 1995 |
| | | | Kamese | MP 6186 |
| | | | Eretmapodites 147 | Prototype |
| | | | Nyando | MP 401 |
| G213-601-567 | 1.0, 2.0 ml. | Polyvalent Patois | Patois | BT 4971 |
| | | | Zegla | BT 5012 |
| | | | Shark River | FE 41 R |
| | | | Mirim | Brain |
| | | | Bertioga | SPAn 1098 |
| G215-701-567 | 1.0, 2.0 ml. | Group Capim | Capim | BeAn 8582 |
| | | | Guajara | BeAn 10615 |
| | | | Bushbush | TR 26668 |
| | | | Acara | BeAn 27639 |
| | | | BeAn 84381 | BeAn 84381 |
| G216-701-567 | 1.0, 2.0 ml. | Group B | Bussuquara | Be An 4073 |
| | | | Dengue-1 | Hawaii A |
| | | | Dengue-2 | TRVL 1751 |
| | | | Dengue-3 | H 87 |
| | | | Dengue-4 | H 241 |
| | | | Edge Hill | C 281 |
| | | | Ilheus | Lemmert |
| | | | Israel Turkey ME | Original |
| | | | JE | Nakayama |

Continued Next Page

| RRB Catalog # | Volume | Type | Viruses | Strains |
|---|---|---|---|---|
| | | | Antigens Used in Preparation of Grouping Fluids | |
| | | Group B (Con't) | Kokobera | MRM 32 |
| | | | Kunjin | MRM 16 |
| | | | MVE | Prototype |
| | | | Ntaya | Prototype |
| | | | SLE | Parton |
| | | | Spondweni | SA Ar 94 |
| | | | Stratford | C 338 |
| | | | Tembusu | MM 1775 |
| | | | Uganda S | Smithburn |
| | | | Usutu | SA Ar 1776 |
| | | | Wesselsbron | SA H 177 |
| | | | WN | E 101 |
| | | | Yellow fever | Asibi |
| | | | Zika | MR 766 |
| | | | Powassan | Byers |
| | | | Louping Ill | OBM |
| | | | Negishi | Prototype |
| | | | Langat | TP 21 |
| | | | Modoc | M 544 |
| | | | Cowbone Ridge | W 10986 |
| | | | USBSG | Burns |
| | | | MML | B 310 A 564 |
| | | | Entebbe bat SG | IL 30 |
| | | | Bukalasa bat | BP 111 |
| | | | Dakar bat | Dak 249 |
| | | | Apoi | Original |
| G219-601-567 | 1.0, 2.0 ml. | Polyvalent Polyam | Palyam | IG 5287 |
| | | | I 68886 | I 68886 |
| | | | IG 15534 | IG 15534 |
| | | | Corriparta | MRM 1 |
| | | | Eth Ar 1846-64 | Eth Ar 1846-64 |
| | | | Eubenangee | IN 1074 |
| | | | Dak Ar B 1327 | Dak Ar B 1327 |
| | | | D'Aguilar | Aus B 8112 |
| G220-701-567 | 1.0, 2.0 ml. | Group Kemerovo | Kemerovo | Rio |
| | | | Chenuda | EgAr 1152 |
| | | | Mono Lake | Cal Ar 861 |
| | | | Wad Medani | EgAr 492 |
| | | | Tribec | Prototype |
| | | | Huacho | Cal Ar 883 |

| RRB Catalog # | Volume | Type | Antigens Used in Preparation of Grouping Fluids: Viruses | Strains |
|---|---|---|---|---|
| G221-501-567 | 1.0, 2.0 ml. | Polyvalent Congo | Congo | Ug 3010 |
| | | | Hazara | UC 280 |
| | | | Ganjam | IG 619 |
| | | | Dugbe | Ibar 1792 |
| | | | Bhanja | IG 690 |
| G222-601-567 | 1.0, 2.0 ml. | Polyvalent 1 | Bahig | Eg B 90 |
| | | | Tete | SA An 3158 |
| | | | Matruh | Eg An 1047-61 |
| | | | Matariya | Eg An 1477-61 |
| | | | | Eg An 1398-61 |
| | | | Burg el Arab | Eg An 3782-62 |
| G223-601-567 | 1.0, 2.0 ml. | Polyvalent 2 | Jurona | Be Ar 40578 |
| | | | Minatitlan | M67u5 |
| | | | MARU 11079 | MARU 11079 |
| | | | Gamboa | MARU 10962 |
| | | | Be An 141106 | Be An 141106 |
| G224-601-567 | 1.0, 2.0 ml. | Polyvalent 3 | Koongal | MRM 31 |
| | | | Wongal | MR 168 |
| | | | Bakau | MM 2325 |
| | | | Ketapang | MM 2549 |
| | | | Mapputa | MRM 186 |
| | | | Trubanaman | MRM 3630 |
| | | | MK 7532 | MK 7532 |
| G225-601-567 | 1.0, 2.0 ml. | Polyvalent 4 | Nyamanini | Eg Ar 1304 |
| | | | Uukuniemi | S-23 |
| | | | Grand Arbaud | Argas 27 |
| | | | Thogoto | IIA |
| G-226-601-567 | 1.0, 2.0 ml. | Polyvalent 5 | Hughes | Dry Tortugas |
| | | | Sawgrass | 64A 1247 |
| | | | Matucare | MARU 24233 |
| | | | Lonestar | TMA 1381 |
| | | | Soldado | TR 52214 |
| G227-601-567 | 1.0, 2.0 ml. | Polyvalent 6 | Marco | Be An 40290 |
| | | | Timbo | Be An 41787 |
| | | | Chaco | Be An 42217 |
| | | | Pacui | Be An 27326 |

| RRB Catalog # | Volume | Type | Antigens Used in Preparation of Grouping Fluids: Viruses | Strains |
|---|---|---|---|---|
| G228-601-567 | 1.0, 2.0 ml. | Polyvalent 7 | Hart Park | AR 70 |
| | | | Flanders | 61-7484 |
| | | | Kern Canyon | M206 |
| | | | Klamath | Microtus 1056 |
| | | | Mt. Elgon bat | BP 846 |
| G229-601-567 | 1.0, 2.0 ml. | Polyvalent 8 | Bluetongue | BT8 |
| | | | EHD | N.J. original |
| | | | Ib Ar 22619 | Ib Ar 22619 |
| | | | Changuinola | BT 436 |
| | | | Irituia | Be An 28873 |
| | | | Colorado tick fever | Condon |
| G230-601-567 | 1.0, 2.0 ml. | Polyvalent 9 | Navarro | Cali 874 |
| | | | Triniti | TR 7994 |
| | | | Aruac | TR 9223 |
| | | | Pan J 19 | Pan J 19 |
| G231-601-567 | 1.0, 2.0 ml. | Polyvalent 10 | Upolu | C5581 |
| | | | DGK | JD 254 |
| | | | Wanowrie | IG 700 |
| | | | Dhori | I 61-13313 |
| G233-601-567 | 1.0, 2.0 ml. | Polyvalent 12 | Okola | YM 50 |
| | | | Olifantsvlei | SA Ar 5133 |
| | | | Witwatersrand | SA Ann 1062 |
| | | | Dak Ar 1569 | Dak Ar 1569 |
| | | | Tataguine | IPD/A 252 |
| G236-601-567 | 1.0, 2.0 ml. | Polyvalent Rabies | Rabies | TR 5843 |
| | | | LCM | Bulgaria |
| | | | Vaccinia | Briody vaccine |
| | | | Newcastle disease | B 12 |
| | | | Herpes | HF |

# REPORT FROM THE INSTITUT PASTEUR & O.R.S.T.O.M.
# BANGUI, CENTRAL AFRICAN REPUBLIC

This report summarizes our results for the year 1973. Investigations have been actively continued in order to collect more information about the life-cycle of sylvatic yellow fever and to obtain a better knowledge of the various other arboviruses in human pathology in Central Africa and reach a better understanding of their epidemiology.

From human sera, taken during the initial phase of acute febrile diseases with or without rash, we obtained one isolate each of ILESHA, TATAGUINE and DUGBE viruses, and two of ZINGA virus. These two isolates of ZINGA confirm the role played in human diseases in Central Africa by this new virus, discovered by DIGOUTTE in 1969 from *Mansonia africana* and *Aedes* gr. *palpalis* and of which one isolate had been obtained in Banqui from a human case in 1971 (see Arbo-Exchange no. 25).

From mosquitoes have been obtained 4 isolates of WEST NILE, one of BAGAZA (local sub-type of NTAYA, prototype strain ArB209), one of MOSSURIL. (Two more isolates are yet to be determined.)

From ticks, on which investigations actively started this year, we have obtained 14 isolates of DUGBE and 8 isolates of JOS viruses, all from *Amblyoma variegatum*. Four more isolates, from *Boophilus annulatus* are still under identification.

Three birds provided us with isolates of INGWAVUMA virus, and WEST NILE was isolated from another.

Results obtained with rodents are interesting; for the first time has been isolated in Central African Republic KOUTANGO virus, from two *Mastomys* and one *Lemnyscomys*.

Catching bats at night with mist-nets was started in 1973 and was actively done during all our field trips; up to now, from about 300 specimens, only one strain of virus has been isolated, yet unidentified.

Serological investigations dealt, as usual, with surveillance of yellow fever in human populations with particular interest, this year, into investigation of the possible yellow fever etiology of the very numerous cases of hepatitis observed in Central Africa: not one single case could be confirmed.

(P. Sureau)

# REPORT FROM THE VIRUS RESEARCH LABORATORY
# UNIVERSITY OF IBADAN, IBADAN
# NIGERIA

1. Virological Studies:

998 blood specimens collected from children with fever reporting sick at the University College Hospital Out-Patient Clinic were sampled for viruses between July and December 1973.

33 isolates were made, 10 of which have been identified: Bwamba/Pongola group (6); West-Nile (2); and Tataguine (2).

2. Yellow Fever Outbreak in South Eastern State, Nigeria:

Reports of clinically suspected cases of Yellow Fever from St. Luke's Hospital, Anua, were received early in December 1973; a Virus Research Laboratory team was dispatched to the area for further investigations. The affected areas are mainly small villages in Uyo and Itu Divisions of the South Eastern State.

Clinical signs seen among hospitalized patients included fever, headache, jaundice, haematemesis and albuminuria. Ten out of eighteen patients admitted in the Hospital died (56% mortality).

Yellow Fever virus isolate was obtained from one of the blood samples collected from febrile patients. Serological studies showed broad Group B virus activity, although some of the sera, especially those from children under 16 years, had homotypic Yellow Fever CF antibodies. Examination of liver specimen from one of the deceased patients was also confirmatory of Yellow Fever infection.

(Akinyele Fabiyi)

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

From July 1 - December 31, 1973, ecological studies on arbovirus with special reference to yellow fever was followed up in our field stations of Bandia and Kedougou. Tick virus research was continued on samples collected at the Dakar Abattoirs.

Mosquito transmission studies have been conducted with yellow fever and other viruses previously isolated from rodents in Dakar: Koutango, Saboya, Yogue, Keuraliba, Toure.

Virological Studies

1.1 Human Blood Samples

437 human blood specimens have been collected from febrile patients in Kedougou and in Bandia. Two virus strains have been isolated, SH 17785 and SH 17783, but are not yet identified.

1.2 Wild Vertebrates Samples

78 specimens were processed for virus isolation. No virus has been isolated from these materials.

1.3 Arthropods

1.3.1 16196 mosquitoes were processed in 276 pools. 3 strains of Bouboui, a group B virus, have been isolated from 3 pools of *Aedes furcifer taylori* collected in Kedougou in October 1973.

5 strains of Zika virus have been isolated from 5 pools of *Aedes furcifer taylori*. One strain of Zika virus has been isolated from a pool of *Aedes dalzieli*, collected in Kedougou in October 1973.

One strain of Sindbis virus has been isolated from a pool of *Aedes vittatus*, one from a pool of *Culex* of the *insidiosus-perfuscus* group, and one from a pool of *Anopheles brohieri*. These 3 strains of Sindbis have been isolated from mosquitoes collected in Kedougou in June and August 1973.

Five virus strains not yet identified have been isolated from pools of *Aedes furcifer taylori* collected in Kedougou in December 1973.

1.3.2 Ticks

8672 ticks were collected at the Dakar abbattoirs and processed in 1616 pools. These specimens yielded 49 strains of viruses:

30 strains of Jos virus from *Amblyomma variegatum*, *Hyalomma truncatum*, *Hyalomma rufipes*, and *Boophilus decoloratus*.
10 strains of CHF-CONGO from *Amblyomma variegatum* (4), *Hyalomma rufipes* (2), *Hyalomma truncatum* (2), *Boophilus decoloratus* (1) and *Hyalomma impressum* (1).

5 strains of Dugbe from *Hyalomma rufipes*, *Hyalomma impeltum*, *Rhipicephalus sulcatus*, *Hyalomma truncatum*, *Amblyomma variegatum*.
2 strains of Bhanja from *Amblyomma variegatum* and *Hyalomma truncatum*.
A pool of *Amblyomma variegatum* showed a mixed infection with CHF-CONGO and Dugbe.
Most of these viruses have been isolated from ticks during the rainy season July to November. During the dry season, few strains are isolated.

## Transmission experiments

*Aedes aegypti* mosquitoes have been fed on an infected animal, usually a suckling mouse at his highest level of viremia.

The first study concerned Koutango, a group B virus isolated in 1968 from the blood of a *Tatera*, trapped in Saboya (Senegal). *Aedes aegypti* are quite readily infected with Koutango virus by feeding on suckling mice with viremia in the range of $10^{4.5}$ to $10^{5.5}$ $LD_{50}$/0.02 ml and are able to transmit virus to newborn mice seven days after the infective blood meal.

The second study concerned two wild strains of yellow fever virus isolated from man during the 1965 epidemic in Diourbel (Senegal). *Aedes aegypti* mosquitoes were almost completely refractory to infection after feeding on suckling mice inoculated with approximately 2,000 $LD_{50}$/0.02 ml ic of yellow fever virus seven days before. *Aedes aegypti* mosquitoes inoculated intra-thoracically with the same strains are readily infected and able to transmit yellow fever virus to suckling mice after an extrinsic incubation period of 7 days.

During these experiments, it was pointed out that suckling mouse, the animal of choice for yellow fever virus isolation, is in fact not highly susceptible. If one infected mosquito is triturated with 1.0 ml of diluent, the suspension does not kill suckling mice inoculated by the ic route. If the volume of diluent is reduced to 0.5 ml, the virus can be recovered in 50% of suckling mice inoculated. Work is in progress to determine the mean virus titers in infected mosquitoes.

(G. Le Gonidec and Y. Robin of the Institut Pasteur; J. Coz, M. Cornet of ORSTOM, Dakar)

## REPORT FROM THE VIROLOGY DEPARTMENT, EGYPTIAN ORGANISATION FOR BIOLOGICAL AND VACCINE PRODUCTION, AGOUZA, EGYPT

Arboviruses.

Attempts of isolations:

a. From ticks: Ticks were pooled according to species, locality of collection, and sex. These pools were inoculated into mice. Out of 210 pools, 5 were positive: TP6, TP24, TP28, TP29 and TP191. TP28 by CFT was found to be related to Tete group which includes Tete, Bahig and matruh viruses. TP191 was found to be negative against the arboviruses we have and lyophilized seed was sent to YARU for identification. TP6 - 24 and 29 are still under study.

b. Blood-sucking insects: These insects were collected by the Faculty of Science, Cairo University, and given to our laboratory for attempts of isolation of viruses. In mice 70 pools of these insects were negative. Other attempts of virus isolations from these insects in tissue culture are under study.

c. Human sera: Blood samples from patients in the Fever Hospitals were drawn during the first three days of fever. Out of 200 cases, 3 were positive; these 3 isolations were identified as Sandfly fever.

Serological survey.

1. Dhori, Wanowrie and Thogato viruses were isolated from ticks infesting camels in Egypt. Attempts to reisolate these viruses from camel and an antibody survey of animal sera, including camels, was started.

2. For infective human arboviruses: We started a survey covering the whole country; animal sera are also included in the tests parallel to the human sera. Tables 1 and 2 show preliminary results. The final report will be written when the study is over.

(Imam Zaghloul Imam)

TABLE I.

Animal Sera Tested by H.I.T.

| Virus Antigen | Total Sera tested | Positive | Titer |
|---|---|---|---|
| Chikungunya | 295 | 3 | 1/20 |
| Semliki forest | 295 | 0 | 0 |
| WEE | 295 | 0 | 0 |
| Sindbis | 295 | 5 | 1/40 |
| Bunyamwera | 295 | 4 | 1/20 |
| Zika | 295 | 10 | 1/80 |
| West Nile | 295 | 36 | 1/80 |

TABLE II.

Human Sera Tested by H.I.T.

| Virus Antigen | Total Sera tested | Positive | Titer |
|---|---|---|---|
| Chikungunya | 260 | 0 | 0 |
| Semliki forest | 260 | 0 | 0 |
| Sindbis | 260 | 18 | 1/40 |
| Zika | 260 | 0 | 0 |
| West Nile | 260 | 91 | 1/80 |
| Langat* | 260 | 28 | 1/40 |
| Bunyamwera | 260 | 4 | 1/20 |

*Antibodies to Langat virus seem to be cross reactive.

REPORT FROM THE ARBOVIRUS UNIT, VIRUS DEPARTMENT
INSTITUTO SUPERIORE DE SANITA, ROME, ITALY

1. Detection of Bhanja virus antigen in cell cultures by fluorescent antibody technique.

The multiplication of Bhanja virus in CV-1 cells was studied staining the viral antigen with the fluorescent antibody technique and measuring the light intensity emitted by the fluorescent cells with a photomultiplier. Very small fluorescent granules appeared in the cytoplasm of the infected cells 9 hours after infection and their intensity reached the maximum 18 hours after infection. Later on, the fluorescence slowly decreased. Signs of cytopathic effect were not observed in the cell sheet until 48 hours after infection.

2. Virus isolations from Phlebotominae.

In the summer of 1971 during field studies on the ecology of arboviruses in several areas of a central Italian region (Toscana), 329 sandflies, mostly females, of a pure colony of Phlebotomus perniciosus were collected in cattle and rabbit stables in Argentario mountain. Sandflies were processed for virus isolation and three virus strains were isolated and reisolated in suckling mice. Isolates were shown to be closely related or identical by CF test.

In order to characterize our sandfly isolates, ultrathin sections of CV-1 cells, infected with the prototype strain (ISS.PHL.3), were observed by electron microscopy. Virions of the average size of 80 nm were observed in the cytoplasm and outside the cell membrane. In order to investigate the pathogenicity of the isolates for laboratory animals, ISS.PHL.3 virus was inoculated intracerebrally in mice, guinea pigs and rabbits. All the animals developed a neurologic disease (flaccid paralysis, convulsions, ataxia, etc.) which was lethal for some of them.

The prototype strain (ISS.PHL.3) reagents were sent to Dr. J. Casals at YARU for identification experiments. From the CF tests performed by Dr. Casals at YARU some conclusions can be derived: (a) ISS.PHL.3 virus is, antigenically, a member of Phletotomus fever group; (b) it is not (nor is it related in the tests to) Sicilian SF, Karimabad, Anhanga, Arumowot, Bujaru, Candiru, Icoaraci, Itaporanga, Punta Toro, Sudan 754-61 and Chagres; (c) it is closely related to Naples SF, but only in one direction.

Tests by immunodiffusion and immunoelectrophoresis are in progress to settle if ISS.PHL.3 is a strain of Naples SF, but somewhat distinct from the prototype Naples, or it is a new virus closely related to Naples SF.

(The field work and the classification of Phlebotominae was done by A. Coluzzi, Institute of Parasitology, University of Rome).

3. Vertebrates as hosts of arboviruses.

3.1 Birds as hosts of Bahig and Matruh viruses. It is known that viruses of the Tete group are transported regularly from Europe to Africa by migrating birds. 1,337 birds (mostly finches) were captured in Italy during their fall migration and Bahig and Matruh viruses were repeatedly isolated (Balducci et al., Annales de Microbiologie, Institut Pasteur, 124 B, 231-237, 1973). The birds studied were only scarcely infested with larval and nymphal stages of ticks.

3.2 Bats. 127 bats (*Myotis myotis*) were captured during the summer of 1972 and bled by cardiac puncture. Pools of brains, and of salivary glands and organs were prepared. Attempts to isolate viruses from blood failed. Isolation experiments from salivary glands and organs are still in progress.

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

REPORT FROM THE DEPARTMENT OF VIROLOGY
UNIVERSITY OF VIENNA, AUSTRIA

## Tick-borne encephalitis

In 1973, a total of 632 cases of TBE were diagnosed in the laboratory, including 9 fatalities. This is the highest incidence of the disease ever recorded in Austria. With the exception of the Tyrol and Vorarlberg, infections had occurred in all provinces of Austria.

During a field study carried out in Switzerland in the spring of 1973 a total of 3070 individuals of Ixodes ricinus were collected in 5 different Kantons where serologic evidence suggested the incidence of TBE. One strain of TBE virus was isolated from ticks collected in a mixed forest (oaks, beech trees and fir trees) near Rheinau, Kanton Zurich.

## Studies on the role of birds as hosts of arboviruses in Central Europe

In Autumn 1971 we started a program concerning the role of birds as hosts of arboviruses endemic in Central Europe on one hand and as possible introducers of arboviruses from tropical and subtropical regions on the other hand. These investigations are still being carried out and will be continued.

All birds were captured with Japanese mist nets in the reed zone of the Neusiedlersee, a lake in the east of Austria. Blood was taken from the jugular vein exclusively. So far blood samples of 1162 birds belonging to 27 species were collected from which 689 were tested for virus and 1148 for hemagglutination inhibiting antibodies against the following antigens: Tick-borne encephalitis (TBE), West Nile (WN), Uukuniemi, Chikungunya, Semliki, Sindbis, Calovo and Tahyna.

From 1148 sera examined in the HI-test, antibodies were found in 94 birds belonging to 12 species. Altogether 104 positive reactions were found: 86 birds had antibodies against only one of the antigens used, 6 sera reacted with two antigens, and 2 sera with three antigens. Most positive reactions were found against Uukuniemi, namely in Locustella luscinioides (1/17), Acrocephalus melanopogon (2/62), A. scirpaceus (6/269), A. arundinaceus (1/61), A. schoenobaenus (1/44), Erithacus rubecula (1/19), Remitz pendulinus (2/77), Parus caeruleus (12/363) and Emberiza schoeniclus (3/135). In addition, hemagglutination inhibiting antibodies were found against West Nile in L. luscinioides (1/15), A. melanopogon (3/62), A. schoenobaenus (1/40), A. scirpaceus (3/243), R. pendulinus (1/77) and P. caeruleus (11/363); against TBE in Porzana porzana (1/1), Tringa ochropus (1/1), L. luscinioides (1/15), A. melanopogon (1/57), A. schoenobaenus (2/40), A. scirpaceus (1/243) and Panurus biarmicus (1/53); against Semliki in A. melanopogon (1/57), A. scirpaceus (6/269), P. biarmicus (1/43) and P. caeruleus (11/363); against Sindbis in L. luscinioides (1/15), A. melanopogon (1/57),

*A. scirpaceus* (2/243), *A. arundinaceus* (1/49), *P. biarmicus* (2/43), *P. caeruleus* (1/363) and *E. schoeniclus* (1/130); and against Calovo in *T. ochropus* (1/1) and *P. caeruleus* (2/363).

So far 62 of the 104 positive reactions in the HI-test were checked in the neutralization test. The results obtained verified the occurrence of antibodies against Uukuniemi in Reed Warbler (*A. scirpaceus*), Robin (*E. rubecula*), Penduline Tit (*R. pendulinus*) and Blue Tit (*P. caeruleus*); against West Nile in Penduline Tit and Blue Tit; against TBE in Moustached Warbler (*A. melanopogon*), Sedge Warbler (*A. schoenobaenus*) and Reed Warbler; against Semliki in Blue Tit; and against Sindbis in Savi's Warbler (*L. luscinioides*).

These results indicate that Uukuniemi virus must occur in Austria. The fact that no antibodies against Tahyna virus could be detected in any of the sera shows again that birds apparently do not play any essential role in the circulation of this agent. It is therefore also doubtful whether the ornitophilic mosquito *Culex modestus* maintains the virus cycle during winter as has been suggested by several authors.

Of particular interest are the serological findings in Bearded Tits, Penduline Tits and Blue Tits. The population of these species found in Austria do not leave Central Europe as a rule (most specimens were caught during winter!); only occasionally single individuals might migrate to Northern Mediterranean regions. The occurrence of hemagglutination inhibiting and of neutralizing antibodies against Semliki in Bearded Tits and Blue Tits gives an important further hint that a group A arbovirus occurs in Europe. Due to the findings of hemagglutination inhibiting and of neutralizing antibodies against West Nile in Penduline Tits and in Blue Tits the possibility must be taken into consideration that West Nile virus occurs (perhaps only occasionally) also in Central Europe.

(Ch. Kunz, H. Aspock, A. Radda)

REPORT FROM THE VIROLOGICAL DEPARTMENT
RESEARCH INSTITUTE OF EPIDEMIOLOGY AND MICROBIOLOGY
BRATISLAVA, CZECHOSLOVAKIA

In a pilot study the efficiency of the lymphatic system in distribution of two different Tahyna virus strains in comparison with Semliki Forest virus in young adult SPF (specific pathogen free) mice shortly after peripheral virus inoculation was studied.

The following virus strains were used: Tahnya virus strain "669", isolated from a young man in 1972 (1), passed twice in suckling mouse brains, and the reference strain "92", isolated from mosquitoes in 1958 (2), passed 6 times in young mouse and 3 times in suckling mouse brains; and a virulent strain of Semliki Forest virus which, in our laboratory, had undergone 1 young mouse and 2 suckling mouse brain passages. Ten percent stock virus suspensions as assayed by intracerebral (i.c.) injection of 8-10 g SPF mice contained $10^{7,5}LD_{50}$ (strain "669"), $10^{7,7}LD_{50}$ (strain "92") and $10^{8,7}LD_{50}$ per 0,03 ml (Semliki Forest virus). It is important to mention that peripheral inoculation of the Semliki Forest virus strain used--in contrast to the Tahyna virus strains--leads inevitably to the development of fatal encephalitis of adult mice within 4-6 days.

Thoracic lymph ducts of three groups each of 6 adult SPF mice weighing 20-22 g were cannulated in their abdominal part by a method described in previous paper (3). The mice were then inoculated into their hind foot pads with the viral strains (10% brain suspensions) and lymph was collected into sterile ampoules (supplemented with 0.1 ml of Puck saline and 3 units of heparin) immersed in an ice bath. Lymph samples harvested at intervals were immediately assayed for infectious virus by i.c. injection of 8-10 g SPF mice. Three similar groups of noncannulated mice were infected as described above and their heart blood was tested at the same time intervals as was the lymph of cannulated animals.

Results of repeated experiments were as shown in Table 1.

TABLE 1.

| Time after inoculation (hours) | Tahyna Virus strain "669" | | Tahyna Virus strain "92" | | Semliki Forest Virus | |
|---|---|---|---|---|---|---|
| | lymph | blood | lymph | blood | lymph | blood |
| 30 minutes | 3/4+ | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 1 hour | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 2 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 3 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 4 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 5 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 6 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |
| 24 hours | 4/4 | 4/4 | 0/4 | 0/4 | 4/4 | 4/4 |

+ = number of dead animals/number of inoculated animals

The obtained data show that the lymphatic system may serve as an efficient canal for quick transport of arboviruses from the site of peripheral inoculation into the central lymph and from there to the blood of adult mice. The difference in spreading of the Tahyna virus strain isolated from man and the strain isolated from mosquitoes in inoculated mice is evident, strain "669" behaving in this respect more like a virulent strain of Semliki Forest virus. It seems that some biological properties of new Tahyna virus isolates from man will differ from those of reference "92" strain and that these new strains might be particularly useful for study of the pathogenesis of Tahyna virus infections.

(Z. Wallnerova)

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## Middelburg virus in mouse organ cultures and peritoneal macrophages

Large (l) and small (s) plaque variants of MID virus were studied, their virulence for mice being slightly different: newborn mice are not killed by i.c. inoculation of MIDs virus after the 2nd day of life; MIDl is virulent for mice up to the 4th to 5th day of life.

After i.p. inoculation in weaned mice, viremia develops for both plaque variants; however, peak titers are reached by MIDl virus 48 hours after inoculation, for MIDs virus 72 hours after inoculation.

For comparison, EMC (picornavirus) was studied.

None of the two plaque variants of MID virus showed any sign of multiplication in cultured mouse peritoneal macrophages. EMC virus multiplied.

MIDs and MIDl multiplied in organ cultures (spleen, liver, brain and muscle) of newborn mice; but compared with MIDl, the replication of MIDs in muscle, spleen and liver started with a 24-hour delay.

Studying organ cultures from mice of increasing age revealed that the organs became refractory to MID virus multiplication when the animals became 10-16 days old.

Replication of MIDs virus in brain from animals aged 2-10 days also started with one-day delay when compared with MIDl virus.

EMC virus multiplied in organs from neonatal, not adult, mice.

Many authors have stressed the importance of macrophages in virus spread, pathogenesis and resistance or susceptibility to viruses. Most studies, however, are based on immunofluorescence tests. Few results are available concerning arboviruses. Johnson (1965) mentions that Sindbis virus did not multiply in macrophages.

Our study showed a parallelism between virulence for mice of EMC and MID viruses and the capacity of the cultured macrophages to support their multiplication. Our results suggest that peritoneal macrophages do not intervene significantly in the spread of MID virus.

The importance of muscle tissue as a replication site for arbovirus was again shown in the experiments with organ cultures. They also show that although there is some correlation between virus multiplication in the intact animal and cell or organcultures derived from it, this correlation is only partial, probably as a result of host defence mechanisms. The two variants of MID virus with different virulence for mice showed corresponding differences in their replication in mouse organ cultures.

(S.R. Pattyn)

REPORT FROM THE LABORATORY OF MICROBIOLOGY, FACULTY OF MEDICINE, UNIVERSITY OF LIEGE, BELGIUM

## Morphological studies of Banzi virus

The H 336 strain of Banzi virus (group B, Flavivirus) has been serially passaged in suckling mice brain. Electron micrographs were taken of negatively stained (PTA), protamine purified, concentrated virus particles. The mean diameter of the complete, spherical, spikes bearing virus particles is 525 ± 5 Å.

The mean diameter is reduced to 380 ± 5 Å if the spikes are not included in the measurements. Some particles display the dumb-bell shape frequently observed with toga-viruses.

The central core (mean diameter of 270 ± 28 Å) seems to have a cubic symetry. All the observed preparations contained many empty envelopes or pieces of envelopes showing the great lability of the Banzi virus structures.

The virus replication in the suckling mouse brain cells was also studied by electron microscopy. One-day-old mice are inoculated intracerebrally. Two days later, at the very late stage of infection, the brains are harvested and fixed. The thin sections showed

- no involvment of the nucleus but a complete disorganization of the cytoplasm, with a mainly perinuclear hypertrophy of the membranous system displaying two typical kinds of structure: numerous small vesicles and a very characteristic series of more or less concentric layer of membranes.
- many complete virus particles (diameter 440 ± 7 Å) with a central core (284 ± 8 Å) located either, isolated, in the extracellular spaces or, in groups, in cells sometimes in small vesicles, sometimes at the center of the membranous spirals.

(C.M. Calberg-Bacq, F. Rentier-Delrue, P.M. Osterrieth)

## REPORT FROM THE DEPARTMENT OF ENTOMOLOGY AND ARBOVIRUS UNIT LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE, LONDON, ENGLAND

Two cell lines established from first stage larvae of *Aedes malayensis* and *A. pseudoscutellaris* are currently at the 86th and 81st subculture level, respectively. WN and JE viruses produce a cytopathic effect in the *A. malayensis* cells. Both these and Dengue-2 virus also produce a cytopathic effect in the *A. pseudoscutellaris* cells. The cytopathic effect in *A. malayensis* is best seen when the cells are grown in glass containers and manifests itself as degenerate clumps of cells and single floating cells. Cells of *A. pseudoscutellaris* line, following infection with the viruses, form syncytia, clearly visible when cells are grown on plastic surfaces (Falcon TC flasks). An unadapted strain of D-2 in infected human serum produced syncytia in the cells. The infected cultures recovered after some days, and formed healthy monolayers. Such cells are being subcultured without syncytia formation, but continue to produce virus. Results with the *A. pseudoscutellaris* cell line are similar to those obtained by Singh et al. (1969) and Chappell et al. (1971) in Singh's *A. albopictus* cells.

Singh, K.R.P. et al. (1969) *Bull. W.H.O.*, 40, 982.
Chappell, W.A. et al. (1971) *Appl. Microbiol.*, 22, 1100.

(M.G.R. Varma and D.I.H. Simpson)

# REPORT FROM THE NATIONAL INSTITUTE FOR MEDICAL RESEARCH
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Cross neutralization studies on 42 flaviviruses (Group B arboviruses) and their respective antisera by a plaque neutralization test in the PS line of pig kidney cells showed that the viruses were divisible into 7 subgroups, except for six viruses which showed no relationship to any other flavivirus. The subgroups were as follows:

Subgroup 1 - Six tickborne viruses: Negishi, Langat, Kyasnur forest disease, louping ill, Omsk and Central European TBE.
Subgroup 2 - Seven viruses associated with bats or small rodents and a further virus isolated from ticks: Apoi, Dakar bat, Entebbe bat, Bukalasa bat, Rio Bravo, Modoc, Cowbone Ridge and Kadam.

(Subgroups 3-7 are all mosquito-borne viruses.)

Subgroup 3 - Japanese encephalitis, Murray valley encephalitis, West Nile, St. Louis, Kunjin, Usutu, Kokobera, Stratford and Alfuy viruses.
Subgroup 4 - Spondweni, Zika and Chuku viruses.
Subgroup 5 - Israel turkey, Ntaya and Tembusu viruses.
Subgroup 6 - Banzi, Uganda S and Edge Hill viruses.
Subgroup 7 - Dengue types 1-4.

Yellow fever, Bussuquara, Wesselsbron, Ilheus, Montana ML and Powassan viruses and antisera showed only homologous reactions.

(A.T. de Madrid and J.S. Porterfield)

REPORT FROM THE DEPARTMENT OF VIROLOGY, CLINIC FOR NERVOUS DISEASES, UNIVERSITY OF COLOGNE, GERMANY

## Intrauterine infection with lymphocytic choriomeningitis (LCM) virus after contact with Syrian hamsters. Report of three cases.

Between 1970 and 1973 51 human cases of LCM infected by Syrian hamsters were observed. In three of these cases an intrauterine infection occurred.

Two women had been in contact with hamsters since the sixth or eighth month of their pregnancy. Their newborn children showed a hydrocephalus internus and on both eyes, the sequelae of chorioretinitis. After drainage of the cerebrospinal fluid the hydrocephali disappeared, but both children develop slower than normally and show severe defects of their vision.

The third case was a 23-year-old woman who noticed slight fever in the seventh week of her pregnancy, 27 days after she had been in contact with a hamster. She developed a biphasic febrile disease with severe headache, but without stiffness of the neck. 28 days after the outbreak of this illness, she started bleeding. At the abrasio two days later the embryo was missing. The placenta appeared normal, but the mucosa showed an infiltration by lymphocytes. CF antibodies against LCM virus were found in the mother's serum and neutralizing antibodies resulted in the following month. From the abrasio material LCM virus was isolated.

In the first case of an intrauterine infection by LCM virus to be observed by KOMROVER et al. (1955), the mother fell ill 12 days before the normal date of delivery; her newborn child fell ill 6 days after birth. They both developed an aseptic meningitis. The child died after an intracerebral haemorrhage which was not contributed to the infectious disease by the authors.

(R. Ackermann)

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REPORT FROM THE ARBOVIRUS LABORATORY, DEPARTMENT OF VIROLOGY
NATIONAL INSTITUTE OF HYGIENE, WARSAW, POLAND

1. Serological survey of foresters in Poland in 1971-1972[1/]

The serological survey was carried out in the population of forestry workers, the group especially exposed to the contact with the virus. The following antigens were used: Polish strain - Kołdobok (for B group); EEE (for A group) and for the first time in Poland - Tahyna (TAH) and Calovo (CVO) viruses. The HI test according to Clarke and Casals (in micromethod of Takatsy) was used in the studies. A total of 21,425 sera was tested. The highest percentage of positive sera had arbo B group antibodies. The percent of positive sera for the forestry workers was 4 times higher than was found in random samples covering the whole population of Poland (see Inf. Exch., No. 17, 1968). The territory of virus dissemination was also found to be much larger than has been previously presented. Some variations concerning different areas were observed; the foresters seem to be a useful group for detecting such variation.

The forestry biotypes in the whole country are potentially areas where tick-borne encephalitis virus can be harbored. For the many endemic districts the percentage of antibodies to TBE virus was about 20%; however, in the Hajnowka district (the oldest known natural focus of TBE virus in Poland) it has attained 40%.

The influence of the age, profession (clerks, forest workers, etc.) and years of work on the seroconversion rate was considered and confirmed in the studies. The possible mechanism of virus spread and arising of new foci were discussed in a forthcoming publication.[2/]

Antibodies to the arbo A group were detected in some northern and central districts (an average between 0-5% were positive).

The occurrence of antibodies to Tahyna and Calovo viruses in Poland was ascertained, mainly in the western part of the country, in territories near the rivers. The average value of positive results was about 2-5%, but in some provinces it was as high as 15-20%. Titers of positive sera were rather low (1/10 - 1/40).

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1/ Supported by programme 05-330-2, PL-480, CDC.

2/ Z. Wroblewska-Mularczykowa, J. Zabicka, E. Nawrocka, D. Olkowska, F.Z. Taytsch-Kapulkin - Serological survey of foresters in Poland in 1971-72, Acta Microbiologica Polonica, 1973 (in press).

2. Attempts to determine genetic characteristics of TBE viruses isolated in Poland

Thirteen strains of TBE virus isolated in different regions of Poland during 1955-1970 and the TP-64 strain of Langat virus were tested for the following genetic markers: pathogenicity for mice by the intracerebral (mic) and subcutaneous (msc) routes of inoculation; antigenic structure (by the grid variant of the HI test); thermal-inactivation rate ($T_{50}^{o}$); resistance to uridine (U); plaque-forming ability; and sensitivity to nonspecific human and animal serum inhibitors.

The influence of some factors, such as the route of virus inoculation, virus passage level, and age of mice on the value of index of infectivity (II) was observed. No fully attenuated strain was found among those investigated; however, some strains were found to have lower pathogenicity for mice and longer incubation periods than other strains isolated from the same region of Poland at the same time.

Slight differences were found also in antigenic structure, sensitivity to nonspecific inhibitors and resistance to physico-chemical factors. No obvious correlation was found between the genetic markers described above and the basic marker of neurovirulence in mice, although in some cases a relative relationship could be observed. More on this problem as well as on the affinity of the strains for the tissue of their natural vector (tick) will be reported after the studies are completed.

(E. Nawrocka)

1. Arbovirus antibodies in milk, a method for epidemiological mapping.

The Inkoo (California group) and Batai (Calovo) antibody content of cattle sera and milk was studied by tissue culture NT. The results of screening (sera in 1:10 dilution, milks 1:2 dilution) are shown below:

| Antibodies in: | Number of Cows | | | |
|---|---|---|---|---|
| | serum + milk + | serum + milk - | serum - milk + | serum - milk - |
| against: | | | | |
| Inkoo virus | 27 | 4* | 0 | 18 |
| Batai virus | 13 | 7* | 1** | 62 |

*titres in sera were low
**a colostrum sample with extraordinary high Inkoo titre

The serum titres correlated with the corresponding milk titres but were about ten times higher. Titres $\geq$10 were also verified by the HI technique.

The milk samples, which are easy to collect, may be a useful substitute for sera in preliminary, large-scale epidemiological mapping. In addition, it is probable that the danger of a milk virus infection is neutralised, if virus-infected milk is mixed with milk from other animals which contains antibodies.

(M. Brummer-Korvenkontio)

2. Inkoo virus antibodies in Finnish Lapps.

The Lapps in Inari commune (69°N, 300 km north of the Arctic Circle) spend a great part of the summer in the open. In tissue culture NT (BHK21 cells) 85% of the sera (170/201) of the adults (20 years) reacted, when the sera were screened in dilution of 1:10, but 99% (198/201) in dilution of 1:2. In the 169 sera of the children (15 years) the titre between 1:2 and 1:10 was encountered only once, in a 10-month-old baby (maternal antibodies?). In the middle-aged group (30 to 49 years) high titres were absent and 20% of the titres were between 1:2 and 1:10. In the old Lapps (over 50 years) there were again some high titres. These results suggest a prolonged but limited persistence of Inkoo virus antibodies and occurrence of reinfections in old age.

(M. Brummer-Korvenkontio)

3. The possibility of the presence of two California group viruses in Finland.

In a study of wildlife sera and blood samples collected from different parts of Finland some of them reacted in the HI test better with LaCrosse than Inkoo haemagglutinin and in tissue culture NT only with the former (to some extent with Tahyna virus, too). This suggests the existence of some other California group virus than Inkoo in Finland.

(M. Brummer-Korvenkontio, P. Saikku, M. Helminen and P. Korhonen)

4. Methodology.

Uukuniemi HA:

The cloned S 23 strain manifests its HA capacity with goose cells at pH's below 5.8, when BHK21 cell culture supernates are used. By concentrating them 10 fold by ultracentrifugation and treating them with tween-ether, hemagglutinins in titres up to 2000 are produced.

(P. Saikku)

Batai (Calovo) NT:

Addition of 5% of accessory factor (fresh guinea pig serum) in tissue culture NT (cells and virus seeded simultaneously) increases titres of mouse hyperimmune ascitic fluids about eightfold and cattle sera about twofold.

(M. Brummer-Korvenkontio)

5. Nephropathia epidemica.

The cases of this haemorrhagic fever reported during the winter of 1973/1974 have occurred in same areas as in the preceding winter, but have shown a tendency to be more severe. A case fatality due to shock in the 3rd day of the illness has been reported.

(J. Lahdevirta)

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Studies with accessory factor

The influence of fresh rhesus monkey serum has been under investigation with regard to its influence on the improvement of the neutralization capacity of antiserum and hyperimmune ascitic fluids (HAF) to arboviruses. All tests were done in flat-bottomed microplates (Limbro Chemical Co.). After incubation of mixtures of serum or HAF and different arboviruses for 1 hour at 37 C in the microplates, VERO cells were added to the wells. End points of virus and antibody titers were calculated on the basis of cytopathogenic effect.

Initially, cross-neutralization tests were performed with viruses of groups A, B and C. Results are shown in Tables 1, 2 and 3. The cross-reactions found were in general the same as when the tests were done in mice. This was clearly demonstrated in the case of group B arboviruses, as seen in Table 2, which compares results of neutralization tests (NT) in mice (i.c.) and in microplates (MNT).

Comparison of MNT results with those obtained by NT in mice or HI is seen in Table 4. Human or animal sera were tested against Mayaro, Caraparu, Oropouche and Piry viruses. In no instance was the MNT less sensitive than the other two techniques in detecting antibodies. In fact the MNT was slightly more sensitive than the HI test for Mayaro, Caraparu and Oropouche viruses. It should be emphasized that these tests were done in the absence of rhesus monkey serum.

Virus titrations in the presence of rhesus monkey serum at a final dilution (FD) of 1:4 (25%) showed lower titers (0.5 to 1,5 log) with Mayaro, Oropouche and Piry viruses than controls. The rhesus serum diluted to 1:8 still reduced the titer of certain viruses (Piry, Ilheus, Turlock, Oropouche, YF, Bussuquara), but not the titer of other viruses such as Mayaro, Mucambo, EEE and Ar 103645. Preliminary results obtained on the influence of rhesus monkey serum (FD 1:8) in the neutralization tests with animal or human sera are as follows:

| | | Homologous titer (neutralization index) | |
|---|---|---|---|
| Virus | Serum or HAF | No Rhesus Serum | Rh Serum Present |
| Mayaro | Human | 1.75 | 3.75 |
| EEE | HAF | 3.25 | 4.25 |
| Mucambo | HAF | 3.5 | 5.75 |
| Ar 103645 | HAF | 2.25 | 4.0 |

Thus, in the presence of rhesus serum all sera or HAF presented a higher neutralization index.

In Table 5 comparisons are made with animal sera in MNT in VERO cells, in the presence or absence of fresh rhesus serum. Caraparu and Apeu viruses were used in the tests. Four- to 12-fold increases in titer were obtained with serum pools 1, 2 and 3, and with serum 1 to Caraparu virus in the presence of rhesus serum; a smaller increase was observed to Apeu virus. Serum 2 (Ma 4184), had a four-fold increase in titer to Apeu virus only, whereas serum pool 4 showed no increase in titer to the two viruses. These sera had an HI pattern to Caraparu virus. The homologous titers of the HAF showed a significantly higher increase than to the heterologous virus, in the presence of rhesus monkey serum.

Results of one experiment designed to determine the lowest dilution of fresh rhesus monkey serum without inhibitory effect on the virus titer, but still capable of improving the neutralization capacity of HAF are shown in Table 6. The Ar 671 (Taiassui) agent, of the Wyeomyia Complex and its homologous HAF were used as a system. It is observed that at a final dilution of 1:32 the rhesus serum had no inhibitory effect on the virus titer, but improved the neutralization capacity of the Ar 671 HAF.

Arbovirus Surveillance on the APEG, Utinga, Catu and Mucambo areas

Surveillance and research activities have continued in these forests, in Belem, during the second half of 1973. Methods of studies rely on the use of sentinel animals, collection of hematophagous arthropods and capture of small mammals. Birds were not netted.

Isolations from sentinel animals: 95 strains were isolated, as seen in Table 7. Most of the isolates (79) came from sentinel mice, the majority of these isolates belonging to the Group C. Sentinel chickens yielded 12 strains of SLE virus, thus bringing to a total of 16 strains of this virus, in the year, isolated from sentinel chickens. Nevertheless, no isolation of SLE virus was obtained from arthropods, but one strain has been isolated from a sentinel monkey in the first half of 1973, with confirmation by serology. Four group C viruses were isolated from sentinel monkeys.

Isolations from wild animals and mosquitoes: these are listed in Table 8. With the exception of one strain of Tacaiuma virus isolated from one *Oryzomys*, all other isolations obtained from small mammals belonged to groups C and Guama. The *Oryzomys* that yielded the Tacaiuma strain was captured at the Utinga forest, Belem, near the Agua Preta water reservoir. The reisolation attempt was positive.

Only 3 isolations were made from mosquitoes, (*Culex*), two of them belonging to the group C and one to the Guama group (An 109303).

One strain of Moju virus was isolated from the blood of a sloth, *Bradypus tridactylus*, captured at the county of Ponta de Pedras, Para State.

## Isolations from Humans

Two isolations were made, one of them being of Oropouche virus (H 244576), from the blood of a laboratory worker (Bel 5279), probably infected when working with this virus. In addition, a strain (H 243004) of Tacaiuma virus was isolated from the blood of a cowboy living at the Paragominas county, Para State. This is the first time that this virus has been isolated from a person, although HI antibodies had been demonstrated among certain populations of the Amazonas region of Brazil, and in French Guiana. He was a 24-year-old male showing fever, headache and dizziness on the day he was bled (August 21); no information was collected on the duration of his illness. He was bled during the course of an investigation of an outbreak of meningococcal meningitis that occurred among road workers, in Paragominas, Para State. He had no demonstrable HI or neutralizing antibodies to Tacaiuma virus in the serum sample taken at the time of the illness; in a second blood sample taken from him on October 11, he had an HI titer of 1:40 to Tacaiuma virus and a neutralization index of 2.5 log to the same agent.

## Serological survey among Koamai Indians

During the course of an investigation on onchocerciasis among the Koamai Indians in December of 1973, 51 individuals were examined for the presence of HI antibodies to arboviruses. The Indian village is located in the north part of the Roraima Territory, close to the boundary with Venezuela. Results are shown in Table 9. High immunity rate was found to Mayaro virus (47%), YF (11%) and Guaroa (9.8%). It should be noted that no antibodies were found to EEE, WEE, Mucambo and SLE viruses.

(Francisco P. Pinheiro, Gilberta Bensabath and Amelia H.P. Andrade)

GROUP A ARBOVIRUSES: CROSS-NEUTRALIZATION TEST IN VERO CELLS (MICROPLATES)

| HAF (FD 1:16) | VIRUSES | | | | | |
|---|---|---|---|---|---|---|
| | Mucambo | Pixuna | Mayaro | Una | EEE | WEE |
| Mucambo | 1,75 * | 0,7 | 0 | ≥ 1,12 | 0,6 | 0,5 |
| Pixuna | 0,5 | 2,7 | 0,12 | 0,12 | 0,37 | ≤ 0,25 |
| Mayaro | 0 | 0 | 2,6 | 1,87 | 0,37 | ≤ 0,25 |
| Una | 0 | 0,45 | ≤ 0,6 | ≥ 5,1 | 0,87 | ≤ 0,25 |
| EEE | 0 | 0 | 0 | 0,12 | 3,6 | ≤ 0,25 |
| WEE | 0 | 0 | 0 | 0,12 | 0,87 | ≥ 5,25 |
| Aura | 0 | 0 | 0 | 0 | 0 | ≤ 0,25 |

* Neutralization index, log 10

GROUP B ARBOVIRUSES: CROSS-NEUTRALIZATION TESTS IN MICE (i.c.)

AND IN VERO CELLS ( IN MICROPLATES )

| HAF (FD 1:16) | | V I R U S E S | | | |
|---|---|---|---|---|---|
| | | YF | Ilheus | SLE | Bussuquara |
| YF | Vero | ≥ 4,0 * | 0,5 | 0,5 | 0,25 |
| | Mice | 4,5 | ≤ 0,33 | 0 | 0,17 |
| Ilheus | Vero | 0,5 | ≥ 5,25 | 0,75 | 0,5 |
| | Mice | ≤ 0,66 | 4,33 | 0,26 | 0,33 |
| SLE | Vero | 0,75 | 1,25 | 3,5 | 0,25 |
| | Mice | 1,0 | 0,67 | 1,5 | 0,33 |
| Bussuquara | Vero | 0 | 0,75 | 0,75 | ≥ 2,75 |
| | Mice | ≤ 0,66 | ≤ 0,33 | 0 | Not Done |

* Neutralization index, log 10

## T A B L E - 3

GROUP C ARBOVIRUSES: CROSS-NEUTRALIZATION TEST IN VERO CELLS ( MICROPLATES )

| HAF (FD 1:16) | VIRUSES | | | | | |
|---|---|---|---|---|---|---|
| | Marituba | Murutucu | Apeu | Caraparu | Oriboca | Itaqui |
| Marituba | ≥6,0 | 4,37 | 1,1 | 0,6 | 0,37 | 0,75 |
| Murutucu | 3,75 | ≥6,37 | 0,35 | ≤0,1 | 1,1 | 0,25 |
| Apeu | ≤1,0 | ≤0,37 | ≥5,6 | 2,37 | 0,37 | 0,5 |
| Caraparu | 2,0 | 1,62 | ≥5,6 | ≥6,1 | 0,62 | ≤1,25 |
| Oriboca | ≤1,0 | ≤0,37 | ≤0,6 | 0,6 | ≥4,87 | 2,0 |
| Itaqui | ≤1,0 | ≤0,37 | 0,6 | 0,37 | 0,37 | 4,25 |

-x- Neutralization index, log 10

T A B L E - 4

COMPARISON BETWEEN IH, NT IN MICE AND MNT IN VERO CELLS

| VIRUSES | Nº SERA TESTED | PERCENTAGE OF POSITIVES | | |
|---|---|---|---|---|
| | | IH | MICE | VERO |
| Mayaro | 96 | 50% | - | 55% |
| Caraparu | 29 | 68% | - | 79% |
| Oropouche | 96 | 75% | - | 81% |
| Piry | 61 | - | 39% | 39% |

EFFECT OF FRESH RHESUS MONKEY SERUM ON THE NEUTRALIZATION CAPACITY OF ANIMAL SERA OR HAF TO GROUP C VIRUSES

| SERUM OR HAF | VIRUSES | | | |
|---|---|---|---|---|
| | CARAPARU | | APEU | |
| | No Rhesus Serum | Rhesus Serum (FD 1:8) | No Rhesus Serum | Rhesus Serum (FD 1:8) |
| Pool 1 ( Ro 12583 e 13120 ) | 1:160 -x- | 1:640 | 1:40 | 1:48 |
| Pool 2 ( Ro 12599 e 12391 ) | 1:160 | 1:2048 | 1:16 | 1:128 |
| Serum 1 ( Ro 12571 (2) ) | 1:40 | 1:160 | < 1:16 | 1:40 |
| Pool 3 ( Ro 12401 (1) e 12781 ) | 1:32 | 1:256 | 1:16 | 1:32 |
| Pool 4 ( Ro 12401 (2) e 13258 ) | 1:160 | 1:160 | 1:40 | 1:40 |
| Serum 2 ( Ma 4184 ) | 1:40 | 1:48 | 1:40 | 1:160 |
| HAF Caraparu | 1:256 | 1:8192 | 1:128 | 1:640 |
| HAF Apeu | 1:32 | 1:160 | 1:192 | 1:2560 |

-x- Serum dilution that neutralized approximately 100 $TCD_{50}$ of virus.

EFFECT OF FRESH RHESUS SERUM ON THE HOMOLOGOUS NEUTRALIZATION CAPACITY OF HAF TO AR 671 VIRUS ( WYEOMYIA COMPLEX )

| DILUTIONS OF HAF | A RHESUS SERUM ( FINAL DILUTION ) | | | | |
|---|---|---|---|---|---|
| | No Rhesus Serum | 1:8 | 1:16 | 1:32 | 1:64 |
| 1:16 | ≥4.0 * | ≥2.5 | ≥3.5 | ≥4.37 | ≥4.25 |
| 1:64 | 3.0 | ≥2.5 | ≥3.5 | ≥4.37 | ≥4.25 |
| 1:256 | 1.75 | ≥2.5 | ≥3.5 | ≥4.37 | 3.25 |
| 1:1024 | 1.25 | ≥2.5 | ≥3.5 | 3.1 | 2.5 |
| 1:4096 | 0.5 | ≥2.5 | 1.5 | 2.37 | 1.75 |
| Titer of HAF ** | 1:640 *** | >1:4096 | 1:2560 | 1:4096 | 1:4096 |
| Virus titer (log 10 $TCD_{50}$/0.075 ml) | 5.5 | 4.0 | 5.0 | 5.87 | 5.7 |

* Neutralization index, log 10

** Highest dilution of HAF that neutralized ± 100 $TCD_{50}$ of virus

*** Titer against 31 $TCD_{50}$

T A B L E - 7

ARBOVIRUSES ISOLATED FROM SENTINEL ANIMALS AT THE APEG FOREST, BELEM, BRAZIL
JULY - DECEMBER 1973

| VIRUSES | SENTINEL ANIMALS | | | Total Number of isolates |
|---|---|---|---|---|
| | Mouse | Chicken | Monkey | |
| SLE ⁎ | | 12 ⁎ | | 12 |
| Caraparu | 22 | | 1 | 23 |
| Itaqui | 15 | | | 15 |
| Murutucu | 7 | | 2 | 9 |
| Oriboca | 4 | | 1 | 5 |
| Gr. C ( untyped ) | 3 | | | 3 |
| Catu | 8 | | | 8 |
| Guama | 10 | | | 10 |
| Moju | 9 | | | 9 |
| Guama Gr. ( untyped ) | 1 | | | 1 |
| TOTALS | 79 | 12 | 4 | 95 |

⁎ Monthly isolations were as follows : July (4) ; August (0) ; September (1) ; October (0) ; November (0) ; December (7).

# TABLE - 8

ARBOVIRUSES ISOLATED FROM WILD ANIMALS AND MOSQUITOES

JULY - DECEMBER 1973 *

| Animal or Mosquitoes | Month | Strain | Viruses |
|---|---|---|---|
| Proechimys | July | An 241044 | Guama |
| Proechimys | July | An 241397 | Caraparu |
| Proechimys | August | An 242318 | Moju |
| Caluromys | August | An 242351 | Marituba |
| Proechimys | August | An 242471 | Caraparu |
| Bradypus tridactylus ** | August | An 243116 | Moju |
| Oryzomys *** | September | An 244235 | Tacaiuma |
| Proechimys | October | An 244866 | Catu |
| Oryzomys | October | An 245034 | Guama |
| Oryzomys | November | An 246546 | Catu |
| Culex sp B nº 19 | June | Ar 238049 | An 109303 |
| Culex sp B nº 27 | June | Ar 238050 | Murutucu |
| Culex (M) taeniopus | October | Ar 246614 | Oriboca |

* included isolations from mosquitoes captured in June

** Captured at Ponta de Pedras county, Para State

*** Captured at the Utinga forest, near the Agua Preta woter reservoir

T A B L E - 9

HI ANTIBODIES TO ARBOVIRUSES AMONG KÔAMAI INDIANS, RORAIMA TERRITORY, DECEMBER 1973

| SEX * | Number of Individuals | GROUP A | | | GROUP B | | | | | OTHER GROUPS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | EEE WEE Mucambo | Mayaro | Cross | YF | Ilheus | Bussuquara | SLE | Cross | Catu | Guaroa | An141106 | Utinga |
| Males | 25 | 0 | 10 | 2 | 3 | 2 | 1 | | 5 | | 2 | | |
| Females | 26 | 0 | 14 | | 3 | 1 | | | 5 | 1 | 3 | 1 | 1 |
| Total | 51 | 0 | 24 | 2 | 6 | 3 | 1 | 0 | 10 | 1 | 5 | 1 | 1 |
| Percentage of Pos | | 0 | 47 | 3,9 | 11 | 6 | 1,9 | 0 | 19 | 1,9 | 9,8 | 1,9 | 1,9 |

* With the exception of 5 males which were under 10 years of age, all other males and females were adults or youngsters

All individuals negative to Caraparu, Itaporanga, Candiru, Tacaiuma and An 174214 antigens.

## 1. EEE Surveillance at Aripo Waller Field

A surveillance programme based mainly on the exposure of sentinel mice was carried out during the year at the A2 station at Aripo-Waller Field. In general, five groups of mice were exposed for 3 nights per week from 19 March to 18 December, 1973.

Between 19 March and the end of July four strains of Guama Group were isolated. Between July and December, 93 isolates were made as shown in the table.

Isolation from Sentinel Mice, Aripo-Waller Field, July - December 1973

| Month | Mouse Groups Exposed | VEE | Guama gp. | Gp.C. | Unid. | Total |
|---|---|---|---|---|---|---|
| July | 15 | | | | | 0 |
| August | 40 | | | | | 0 |
| September | 60 | | 1 | | | 1 |
| October | 55 | 9 | 18 | 2 | 3 | 32 |
| November | 60 | 49 | | | | 49 |
| December | 25 | 11 | | | | 11 |
| | 225 | 69 | 19 | 2 | 3 | 93 |

Because the programme was intended to monitor the presence of EEE, specific identifications were only made for Group A isolates.

In addition to the sentinel mice, 6334 mosquitoes (23 species) from mouse-baited traps were inoculated into mice in 259 pools. Only one strain of VEE was isolated from *Culex portesi*. Another 4638 resting female mosquitoes, when inoculated into mice, yielded a single strain of VEE from parous *C. portesi*.

As an experiment, 9 *Didelphis* opossums were exposed for 28 nights as sentinel animals. 3 Guama Gp., 2 Gp.C and one VEE isolates were made from these animals.

EEE was not isolated and one may speculate as to whether the very severe and prolonged dry season (February-July instead of February to May) might have been responsible in some way.

## 2. Viruses isolated from bats

Two viruses, both new at least for Trinidad, were isolated from insectivorous bats in 1973. One, a group B arbovirus was isolated from salivary glands and oral swabs from *Molossus ater*. The other was isolated from a brain-salivary gland pool from *Chilonycteris rubiginosa*.

(C.O.R. Everard, J.B. Davies, J.L. Price and M.C. Williams)

## Epizoodemic of VEE in the Venezuelan Guajira, October-November 1973

On September 4, 1973, an alert was sent to the health services in the districts of Paez and Mara of Zulia State, Venezuela (Fig. 1), to maintain surveillance of the number of patients seen each day and to take blood samples of all febrile children under ten years of age. The alert was issued after a research group studying Venezuelan equine encephalitis (VEE) reported that hamsters used as sentinels in the possible endemic focus of VEE in that area were dying and because the mosquito population was increasing after heavy rains.

From October 8, a rise was observed in the number of people attending the Rural Health Center. By October 10, there were reports, which were not confirmed, of deaths in equidae. In a visit made that day to the affected area, several dead burros were observed, and the Veterinary Health Center confirmed the suspects.

On October 12, the first neurologic case was presented in humans in Paraguaipoa. On the 15th day the laboratory confirmed the presence of the VEE virus in human beings.

From that day onwards, aerial and terrestrial spraying of insecticides started. A rapid decrease of the population of mosquitoes was observed; and consequently a decrease in number of human cases occurred and the immediate disappearance of the cases in equidae, despite the fact that, after a short recess, the rain continued to fall and flood the area, with reappearance of the mosquito population.

It was considered that the epidemic reached the Guajira and Sinamaica municipium of the Paez District, and Luis de Vincente of the Mara District. In the latter district there were no cases reported in equidae, and the distribution of human cases by age did not coincide with that of the Paez District since 45% of the cases were present in people older than 20. It was included in the outbreak due to the presence of 4 cases really neurologic, of which one died (Table 1).

On clinical grounds three deaths were attributed to VEE, two from the Guajira Municipium, 1 and 3 years of age, and the third from the Luis de Vincente Municipium, 2 years of age. No virus was isolated from samples of serum of these children. The Sinamaica Municipium did not register deaths.

The epidemic was considered to have ended around November 20.

Although there was an increase in the number of patients in the San Rafael and Islas de Toas and San Carlos Municipium, they were not included in the epidemic by the total absence of the neurologic cases, and the negative

report of the equine cases. However, only the laboratory results will be able to tell us what really happened in these populations. Some of the cases presented with exanthema which led us to suspect dengue.

We have no data about morbidity and mortality in equidae.

Isolation and serology. From 40 human samples processed in the Instituto de Investigacion Clinica during the outbreak, the virus was isolated and identified in 7 of them, all being from children under 5 years of age belonging to the Paez District. In the same manner, an agent was isolated and identified as VEE from an apparently health horse in the Guajira Municipium.

The Instituto Nacional de Higiene (INH) tested 94 human samples and has confirmed the presence of the agent in 9 of them. Another 11 are being kept for confirmation.

The Instituto Venezolano de Investigaciones Cientificas (IVIC) has processed 487 human samples and has achieved 152 isolations, from which 10 have been confirmed as VEE. In the same way, 47 isolation were obtained from 318 equidae samples.

In December 1973, 28 second samples in humans were obtained in the San Rafael Municipium and a conversion for VEE in HI in a seven months baby girl was demonstrated. The epidemiologic investigation showed that the girl had not travelled to the known epidemic area in the last months. This seems to confirm the extension of the epidemic to the San Rafael Municipium.

The final report about the epidemic is not yet finished.

Vaccination. Commencing on November 22, 1973, a vaccination campaign was started with the live attenuated vaccine TC-83. The areas to be immunized include those considered of high risk when the rain begins again during the middle of this year.

(Slavia Ryder)

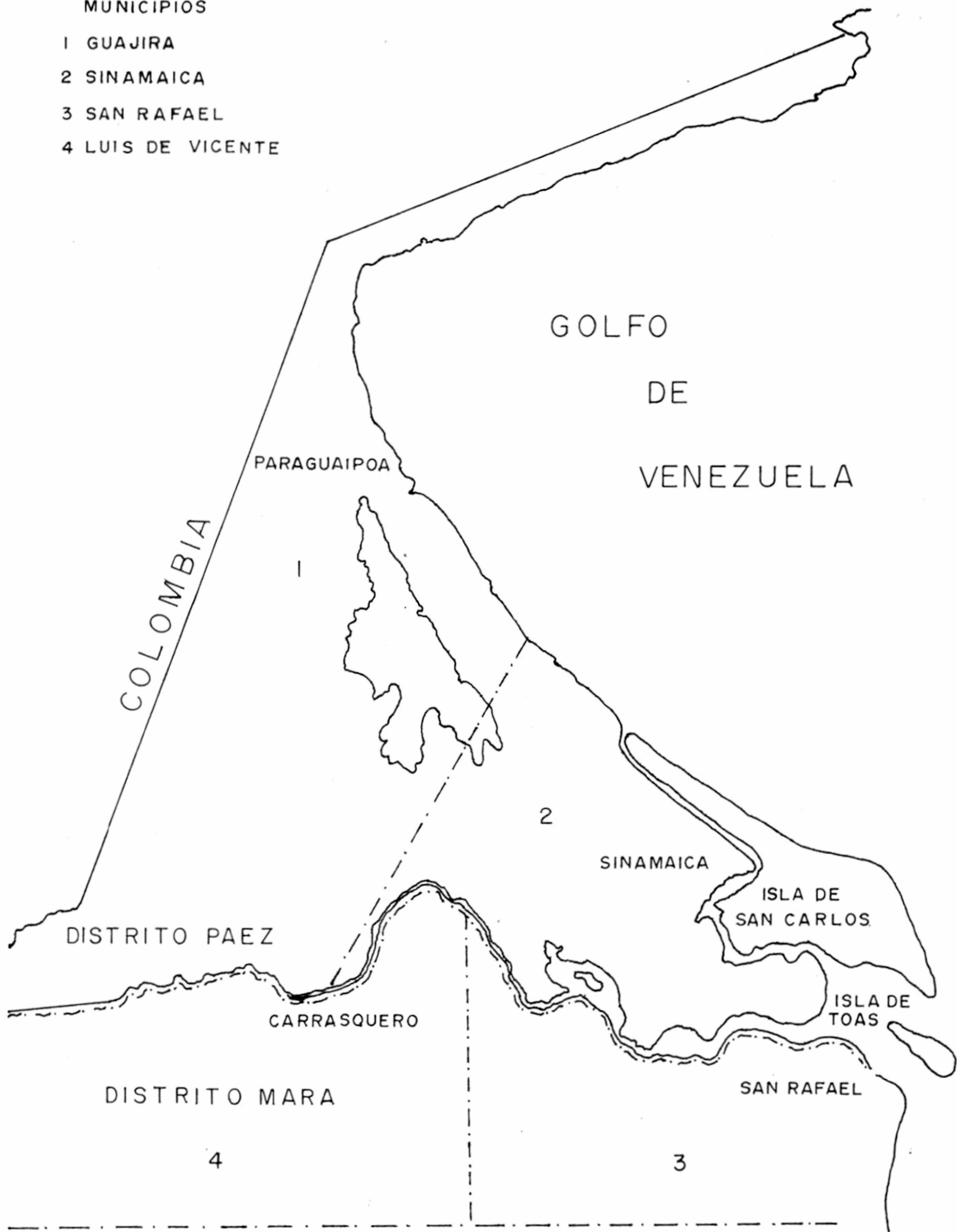

MUNICIPIOS
1 GUAJIRA
2 SINAMAICA
3 SAN RAFAEL
4 LUIS DE VICENTE
GOLFO
DE
VENEZUELA
PARAGUAIPOA
COLOMBIA
1
2
SINAMAICA
ISLA DE
SAN CARLOS
ISLA DE
TOAS
DISTRITO PAEZ
CARRASQUERO
SAN RAFAEL
DISTRITO MARA
4
3

TABLE 1

| Health Center | Cases | Neurologic | Deaths |
|---|---|---|---|
| Paraguaipoa | 435 | 38 | 2 |
| Sinamaica | 337 | 8 | 0 |
| Carrasquero | 45 | 4 | 1 |
| Total | 817 | 50 | 3 |

REPORT FROM THE HEALTH INSTITUTE OF COLOMBIA
ARBO-VIRUS SECTION, BOGOTA, COLOMBIA

## Summary Report for 1973

During 1973, the Institute continued conducting active surveillance in the fields of yellow fever, Venezuelan equine encephalitis and dengue. Research programs were continued in special areas, attempting to elucidate certain viral etiological unknowns, especially with regard to ecological variations.

### 1. Yellow fever

During 1973, yellow fever made one of its cyclic visits to that part of the forest periphery corresponding to Eastern Colombia, at the zone of transition between the Amazonian rain forest and the grassy plain or savannah, called the Eastern Llanos. This part of the country has been the theater of abundant colonization during the last decade. There are several new towns, whose founders eak out their existence from trade, cattle breeding, timber exploitation and plantations in the gallery forests of the Ariari, Guejar and Guaviare rivers and their affluents. The mass invasion by susceptible new-comers into areas where the vector is prevalent and where there is periodic yellow fever virus activity offers the conditions for the outbreaks of the disease. Were it not for the vaccine, we would have witnessed this year a repetition of past episodes with high mortality, because the virus, in its endless trajectory throughout the Amazon Basin hit this year Mapiripan, on the Guaviare bench. Positive livers were detected there by the viscerotomy service, from December 1972 to April 1973.

The vagaries of yellow fever virus were further revealed by the incidence of positive cases in the Sarare plains, north along the eastern cordillera foothills. These occurred at the headwaters of the Arauca river in June and July 1973. The virus route was probably up the Arauca gallery-forest. Had it taken the old, well known, path of the foothills, by San Martin, Guamal, Acacias, Restrepo and Cumaral, heavily populated as it is today, the number of cases would have been heavier, in spite of vaccination. It is possible that the virus did not follow the usual pathway because of the extensive forest destruction by colonizers.

In the Middle Magdalena Valley, a few yellow fever cases occurred in San Luis, Antioquia. The interesting feature of this small outbreak lies in the recurrence of yellow fever in a zone of old colonization, with the pristine environment devastated and the contact of humans with the original ecology much reduced; here, also, the vaccine must have had a great influence on incidence.

### 2. Venezuelan Equine Encephalitis

During 1973 the main activities are summarized as follows:

2.1 Studies continued to be conducted in the sylvatic focus of Puerto Boyaca where previous investigation started in May 1970 had shown a remarkable permanence of the virus there. The virus was again active in the area during 1973. It was possible to classify three strains isolated from Puerto Boyaca: all were identified as I-D, and when inoculated into susceptible horses, by the subcutaneous route, did not produce apparent clinical disease in them.

2.2 Surveillance, particularly by means of sentinel hamsters, was made of the localities of Armero and Ambalema, an area mainly dedicated to rice and cotton growing as well as cattle breeding. No evidence of viral activity was detected in these places located one hundred kilometers south of the Puerto Boyaca focus.

2.3 Studies, including careful observation of *Deinocerites*, were conducted in Guajira, a peninsula which has a long record of periodic outbreaks, in order to find out whether there was any viral activity during the inter-epidemic periods. No such an activity was demonstrated during 1973.

2.4 Prior to 1973, the last known epidemic in the semi-desert country of Guajira occurred during 1970. It was speculated that during 1973 an outbreak was likely to occur because of the increase of susceptible equines and in case there were a rainy season intense enough to allow for a sufficient multiplication of mosquitoes. During 1973, the rains were heavier than in previous years (in 1972 there was no rain at all) and started in July; October is the usual month for the beginning of the rainy season. As expected, an outbreak, confined mainly to Pajaro, near Riohacha, occurred from late September to the first two weeks of October. About 200 horses died, and the virus was isolated, particularly from apparently healthy horses grazing in the vicinity of the sick ones. The episode was probably linked to the epidemic observed simultaneously in neighboring Venezuela.

2.5 Particular attention was given to the study of the different outbreaks of febrile disease in the country. This permitted the identification of an epidemic of VEE in the village of Lozania, on the shore of the artificial lake of the Prado dam, in Tolima. Special studies are conducted in the area because of the ecological changes produced by the dam and by the rather strange fact that nobody in the village complained of having lost horses.

3. Dengue

Surveillance of dengue continued in Cartagena by means of the study of sentinel populations, which still showed there some viral activity early in the year in spite of the fact that *A. aegypti* numbers had been substantially reduced.

The study of outbreaks of febrile disease in the Atlantic Coast permitted the identification of dengue epidemics in Sabanagrande during June and in Gaira During July 1973, at a time when both places were still heavily infested with the vector. These towns, as a number of other localities in the aforementioned general area, had been spared by the wave of 1971-1972. No evidence of dengue hemorrhagic fever was observed.

(Hernando Groot)

REPORT FROM THE VIROLOGY DEPARTMENT, NATIONAL INSTITUTE OF HYGIENE "LEOPOLDO IZQUIETA PEREZ", GUAYAQUIL, ECUADOR

Isolations of VEE virus from vertebrates, Ecuador, 1973.[1]

| Species | Date Exposed | Date of Sample | Location | Specimen | Comments |
|---|---|---|---|---|---|
| Hamster | 2/16-26 | 2/26 | Jose Reyes farm, Ayangue. | Blood | |
| Hamster | 2/16-26 | 2/26 | Jose Reyes farm, Ayangue | Brain | |
| Hamster | 3/19-30 | 4/ 2 | El Papagayo farm, Babahoyo-Quevedo. | Blood | |
| Hamster | 3/19-30 | 4/ 2 | Beata Elvira farm, Babahoyo-Quevedo | Visceral pool | |
| Human Being | | 4/ 6 | Palmar | CSF | Sick: fever, headache, convulsions, vomiting |
| Horse | | 5/ 6 | Guayaquil | Serum | |
| Human Being | | 6/7 | Virus Lab | Blood - Throat Swab | Laboratory infection |

[1] All isolations were made by intracerebral inoculation of suckling mice.

(Ernesto Gutierrez Vera)

REPORT FROM THE CENTRO NACIONAL DE DIAGNOSTICO Y REFERENCIA
INSTITUTO DE SALUBRIDAD Y ENFERMEDADES TROPICALES, MEXICO 17, D.F.

HI antibody titers against VEE and SLE viruses in sera submitted to the Centro Nacional de Diagnostico y Referencia

| No. of human sera | Locality | Titer* VEE | SLE |
|---|---|---|---|
| 2 | Mexico City | n** | n |
| 7 | Huitzuco Gro. | n | n |
| 1 | Huitzuco Gro. | 10 | n |
| 1 | Huitzuco Gro. | 40 | 10 |
| 1a*** | Huitzuco Gro. | 80 | n |
| 1b | Huitzuco Gro. | n | n |
| 1a | Huitzuco Gro. | 80 | n |
| 1b | Huitzuco Gro. | 80 | n |
| 3 | Misantla Ver. | n | n |
| 3 | Aguascalientes Ags. | n | n |
| 2 | Aguascalientes Ags. | 80 | n |
| 1 | Aguascalientes Ags. | 20 | n |
| 1 | Aguascalientes Ags. | 160 | n |
| 1c | Guanajuato Gto. | n | n |
| 1a | Los Mochis Sin. | 20 | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | 160 | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | n | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | 20 | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | 80 | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | 40 | n |
| 1b | Los Mochis Sin. | 40 | n |
| 1a | Los Mochis Sin. | 80 | n |
| 1b | Los Mochis Sin. | n | n |
| 1a | Los Mochis Sin. | 40 | n |
| 1b | Los Mochis Sin. | 40 | n |

* Reciprocal of the highest dilution of the serum

** n = negative

*** 1a are sera from women who had encephalitis some time during pregnancy. After delivery mothers and products were bled and and tested for VEE antibody; 1b are sera from the products.

(David Bessudo M. and Maria L. Zarate)

# REPORT FROM THE SAN JUAN TROPICAL DISEASE LABORATORY
## CDC, SAN JUAN, PUERTO RICO

The following are significant developments in Puerto Rico since the last issue.

1. A few cases of dengue have continued to occur in Villalba through November and December 1973 and into January 1974, following mosquito control activities by the Puerto Rico Health Department.

2. New foci of confirmed dengue activity have been identified in Santa Isabel, Hormigueros, Guánica (site of a large outbreak in 1972) and San Sebastian. As of February 1974, only the towns of Guayanilla and Santa Isabel are reporting suspect cases. The attached figure shows towns with confirmed dengue since 1970.

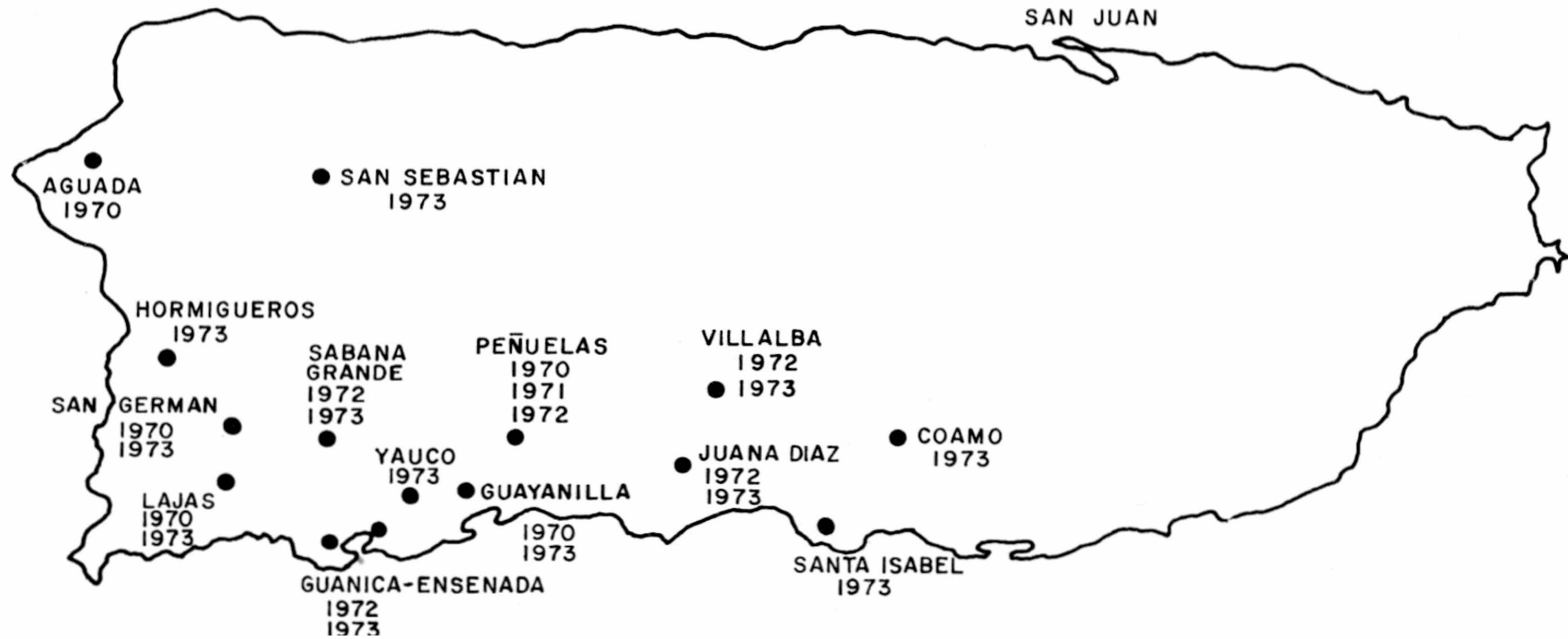
3. No hemorrhagic and/or shock-like manifestations of dengue have been observed or reported. Between 20 and 30% of confirmed cases exhibit a secondary type antibody response.

4. Surveillance activities have been expanded and now include health centers on all parts of the Island.

5. Miss Gladys Sather, formerly Chief, Arbovirus Reference Unit, CDC, Atlanta, jointed the staff of our Virology Unit in November 1973.

(B.L. Cline, W.T. Rymzo, Jr., G.E. Kemp, and G.E. Sather)

# TOWNS WITH CONFIRMED DENGUE - 2 IN PUERTO RICO DURING 1970 THROUGH DECEMBER 1973



REPORT FROM THE DEPARTMENT OF MEDICINE
CEDARS OF LEBANON HOSPITAL, MIAMI, FLORIDA

Study of Dengue Fever in the Caribbean

In an attempt to determine the incidence of dengue infection in the capital of the Dominican Republic, Santo Domingo, triple blood specimens from 198 children between one and 12 years of age were collected from a densely populated, northwest barrio, Los Minas, between November 1972 and May 1973. Results of HI tests using dengue 2 and dengue 3 antigens on these 198 triplicate samples demonstrated 4 seroconversions and 33 four-fold rises in titers (Table 1). This is an infection incidence of 13.5% in 19 to 25 weeks. Eleven serodiagnostic rises, 3 in age group 1 to 5 and 8 in age group 8 to 12 years, were observed during November 1972 to February 1973, while 22 such changes in titers were found during February 1973 and May 1973, two in age groups 1 to 5 and 20 in age group 6 to 12 years. These serologic changes were observed in spite of an 89% (42/47) HI dengue antibody prevalence in the children ages 1 to 5 years, and 98% (148/151) antibody prevalence for specimens from the children ages 6 to 12 years (Table 2).

In addition, forty-seven pairs of serum from pregnant women experiencing uterine hemorrhage were tested and compared with 92 pairs from "normal" pregnant women of the same age, socio-economic status from the same hospital. These women came from several parts of Santo Domingo. 44% (21/47) of the hemorrhagic cases showed serodiagnostic rises to dengue 2 and/or dengue 3 antigens in HI tests, while 34% (31/92) of the non-hemorrhagic or control group seroconverted or had 4 fold HI antibody rise. The difference was not significant ($P>.02$). As a combined group these pregnant women had an HI antibody prevalence rate for dengue of 94% and a serologic infection rate of 37% during an average of six weeks (Table 3). This indicates a weekly infection rate of approximately 6%. Also, from the above-mentioned hospital, 26 of 27 cord bloods were HI positive to dengue 2 and/or dengue 3 antigen (Table 4).

These data indicate widespread continuing group B arbovirus infections in Santo Domingo.

(A.K. Ventura and J.J. Ehrenkranz)

TABLE 1

Dengue diagnostic serology on triplicate blood specimens collected from 198 Los Minas children between November 1972 and May 1973.

| Age groups in years | No. of children tested | Conversions and HI titers* to the following antigens: Dengue 2 only | Dengue 3 only | Dengue 2 & Dengue 3 | Totals |
|---|---|---|---|---|---|
| 1- 5 | 47 | 3 | 0 | 4 | 7 |
| 6-12 | 151 | 5 | 5 | 20 | 30 |
| TOTALS | 198 | 8 | 5 | 24 | 37 |

*Four-fold rises

TABLE 2

Dengue HI in Los Minas children - November 1972

| Age in years | Number Tested | Number ≥ 1:10 to following antigens: Dengue 2 only | Dengue 3 only | Dengue 2 & 3 | Percent Positive Dengue 2 and/or Dengue 3 |
|---|---|---|---|---|---|
| 1- 5 | 47 | 3 | 2 | 37 | 89* |
| 6-12 | 151 | 2 | 3 | 143 | 98 |
| TOTALS | 198 | 5 | 5 | 180 | 96 |

*Similar positive precentages were obtained for sera collected in February and May 1973 for the same individuals.

TABLE 3

Diagnostic dengue serology of pregnant women from Santo Domingo during January to October 1973*

| Age in years | Number Tested | Conversions** and 4-fold rises in HI titers $D_2$ | $D_3$ | $D_2$ & $D_3$ | Totals | Infect. Rate |
|---|---|---|---|---|---|---|
| 15-30 | 108 | 7 | 8 | 25 | 40 | 37% |
| 31-50 | 31 | 6 | 1 | 5 | 12 | 39% |
| TOTALS | 139 | 13 | 9 | 30 | 52 | 37% |

*Average time between specimens in a pair was 6 weeks.
**6 seroconversions were noted in the 6 negative individuals that were included in this study.

TABLE 4

Serology of cord bloods and respective mothers' sera from Santo Domingo

| Serums | Tested / Positive | HI Positives $\geq$ 1:10 Dengue 2 only | Dengue 3 only | Dengue 2 & 3 |
|---|---|---|---|---|
| Cord | 26/27 | 2 | 0 | 24 |
| Mothers | 24/27 | 3 | 1 | 20 |

REPORT FROM THE FLORIDA DIVISION OF HEALTH
BUREAU OF LABORATORIES
JACKSONVILLE, FLORIDA

Human and Animal Sera Screened
by the HI Technique with Arbovirus Antigens*

January 1973 - December 1973

| Species | Number of Sera | Reactors |
|---|---|---|
| Humans | 516 | (1) 3 EEE<br>(2) 1 Dengue II |
| Horses | 149 | 65 EEE<br>37 WEE<br>30 VEE |
| Field Specimens (mammals)<br>Sentinel Fowl Sera | 796 | (3) 28 VEE |
| Total | 1461 | 164 |

1) 2 children (living with brain damage).
   1 child (death), brain shipped directly to CDC.
2) Adult male, infected in Haiti.
3) Field specimens, South Florida study areas where VEE (FE3-7C) is endemic.

*Arbovirus Antigens:
EEE - Eastern Equine Encephalitis
WEE - Western Equine Encephalitis
SLE - St. Louis Encephalitis TBH-28
VEE - Venezuelan Equine Encephalitis TC-83 and/or FE3-7C

(Elsie E. Buff)

REPORT FROM THE DEPARTMENT OF VIRUS DISEASES
DIVISION OF COMMUNICABLE DISEASE AND IMMUNOLOGY
WALTER REED ARMY INSTITUTE OF RESEARCH, WASHINGTON, D.C.

During arbovirus ecology studies on the eastern shore of Maryland the Keystone strain of California encephalitis virus was repeatedly isolated from *Aedes atlanticus* mosquitoes. The field infection rate from this species has remained approximately the same, both between years and during each summer studied, even during periods when large numbers of adults were emerging, suggesting the possibility of virus maintenance through transovarial transmission. Further, virus was isolated from among the very first adult *A. atlanticus* collected and tested, suggesting that these mosquitoes had emerged already infected. To demonstrate the suspected transovarial transmission of this virus, developmental stages of *A. atlanticus* were collected from the field. Larvae were identified and pooled for virus isolation attempts in suckling mice. Pupae were allowed to emerge in the laboratory, then were segregated by sex and pooled for virus isolation attempts. Keystone virus was isolated from larvae, reared males and reared females, unequivocally demonstrating transovarial transmission of the Keystone strain of California encephalitis by *A. atlanticus*.

(James W. LeDuc)

Keystone Virus from Pocomoke Cypress Swamp, Maryland (1971 - 1973)

| No. of Strains | No. mosq. tested | Isolated from | Date Collected |
|---|---|---|---|
| 2 | 204 | A. atlanticus | June 71 |
| 22 | 6,601 | A. atlanticus | July 71 |
| 6 | 1,108 | A. atlanticus | Aug 71 |
| 2 | 393 | A. atlanticus | Sept 71 |
| 52 | 18,333 | A. atlanticus | July 72 |
| 1 | 518 | A. atlanticus larvae | Aug 73 |
| 5 | 2,040 | A. atlanticus males* | Aug 73 |
| 3 | 1,688 | A. atlanticus females* | Aug 73 |

*Laboratory reared adults from field collected pupae

REPORT FROM THE DEPARTMENT OF PATHOBIOLOGY
THE JOHNS HOPKINS UNIVERSITY SCHOOL OF HYGIENE AND
PUBLIC HEALTH, BALTIMORE, MARYLAND

An outbreak of an illness characterized by fever, anorexia, malaise and jaundice and diagnosed clinically as infectious hepatitis occurred in Kathmandu, Nepal, from February through October, 1973. During this period, 637 such patients were admitted to civilian hospitals, including 115 pregnant women. Mortality in hospitalized patients was less than 1% except in the pregnant women, of whom 41 (36%) went into coma and 31 (27%) died. Survey data indicate that total incidence exceeded 10,000 cases in Kathmandu Valley (population about 600,000).

Although hepatitis occurs frequently in Western foreigners visiting Nepal, this is the first record of an epidemic in the indigenous population. Peak incidence was in young adults, who accounted for the majority of hospital admissions. Injections were ruled out as a source of infection. Sewage disposal throughout the valley is generally inadequate and fecal contamination of the environment is evident, but no common source for the epidemic could be located. High incidence occurred in areas supplied by several independent water sources. There was no increase in hepatitis cases in foreigners, most of whom received gamma globulin either before or during the epidemic.

Immunodiffusion testing for Australia antigen was negative in all of 53 acute patient sera tested (confirmed courtesy of Dr. R.W. McCallum of Yale University School of Medicine). Complement fixation on sera from 23 patients was done by Dr. R.E. Shope, Yale Arbovirus Research Unit, Yale University School of Medicine, using the following antigens: chikungunya, Ross River, dengue-2, yellow fever, Omsk hemorrhagic fever, Kyasnur Forest disease, Zika, Crimean hemorrhagic fever-Congo, Junin, and Lassa. Results were negative with sera in dilutions 1:4, 1:8, 1:16, and 1:32. One serum reacted with all antigens by CF, these reactions being considered non-specific.

(A.B. Hillis and F.B. Bang)

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

Surveillance for arbovirus infections in New Jersey involved collection and testing of mosquitoes from known EE endemic areas, and virus isolation and serological testing of suspect infections in pheasants, pigeons and equines. Activity of both EE and WE viruses was detected, but only EE was related to disease.

Four human infections of EE were documented: one fatal case in an adult female, with symptoms including fever, headache, encephalitis and upper respiratory infection, from whose brain the virus was isolated; one infection of a male infant less than one year of age with neurologic sequelae, suffering fever, encephalitis, and upper respiratory symptoms, with four-fold rises in sera by HI and CF; and two recovered cases, one a young adult female and the other a male more than 60 years of age, both confirmed by four-fold serologic rises in titer of CF, HI, and (for the younger person) neutralizing antibody titers.

Tabulations of serologic and isolation results for the animal and mosquito studies are shown in the following three tables.

(Martin Goldfield)

Isolation and/or Serology of Animal in New Jersey

| Location | Animal Species | Date Specimen Collected | Isolation Data | |
|---|---|---|---|---|
| | | | Specimen | Virus |
| Fort Dix | Pheasant | 8/1/73 | Allantoic fluid | EE |
| Cape May | Pigeon | 8/16/73 (rec'd) | Allantoic fluid | EE |
| Medford | Pheasant | 8/30/73 | Brain | EE |
| Sussex | Pheasant | 9/19/73 | Allantoic fluid | EE |
| Sussex | Pheasant | 10/4/73 | Brain | EE |
| Forked River | Pheasant | 3rd week of August | Brain | EE* |

*results obtained from Yale

| LOCATION | ANIMAL SPECIES | DATE SPECIMEN COLLECTED | Isolation Data | | Serology Data | | | PATHOLOGY |
|---|---|---|---|---|---|---|---|---|
| | | | Organs | Blood | HI | CF | Neut. | |
| Vincentown<br>Burlington County | Equine | 7-29-73 | No Brain<br>Submitted | Negative | B1<br>640 | Unsat. | 1.7 | No Brain<br>Submitted |
| Jobstown<br>Burlington County | " | 8-1-73<br>8-3-73 | Brain-EE | Negative | B1<br>640<br>B2<br>640 | <8<br><br><br><8 | <0.6<br><br><br><0.6 | Positive |
| Mullica Hill<br>Gloucester County | " | 8-22-73 | Brain-EE | Negative | B1<br>2560 | ND | 1.5 | Positive |
| Cape May<br>Cape May County | " | 8-25-73 | Brain-EE | Negative | B1<br>1280 | <4 | 2.2 | Positive |
| Cumberland<br>Cumberland County | " | 8-25-73 | No Brain<br>Submitted | Negative | B1<br>640 | ND | 3.4 | No Brain<br>Submitted |
| Monroeville<br>Salem County | " | 8-27-73 | Brain-EE | Negative | B1<br>640 | <4 | 2.7 | Positive |
| Lakewood<br>Ocean County | " | 8-27-73<br>8-28-73 | No Brain<br>Submitted | Negative | B1 ≥ 10,240<br>B2 ≤ 10,240 | 32<br>64 | ND<br>ND | No Brain<br>Submitted |
| Nesco<br>Atlantic County | " | 8-29-73 | Brain-EE | Negative | B1<br>1280 | Unsat. | 2.8 | Positive |
| Dorothy<br>Atlantic County | " | 9-4-73 | Brain-EE | Negative | B1<br>320 | Unsat. | 2.6 | Positive |
| Elmer<br>Salem County | " | 9-6-73 | Brain-EE | Negative | B1<br>320 | ND | <1.2 | Positive |
| Egg Harbor<br>Atlantic County | " | 9-6-73 | Brain-EE | Negative | B1<br>320 | Unsat. | 1.8 | Positive |
| Delaware * | " | 9-7-73 | Brain-EE | No blood<br>Submitted | --- | --- | --- | Positive |
| Bricktown<br>Ocean County | " | 9-7-73 | Brain-EE | Negative | B1<br>2560 | <4 | ND | Positive |
| Salem<br>Salem County | " | 9-12-73 | Brain-EE | Negative | B1<br>2560 | Unsat. | ND | Positive |
| Morganville<br>Monmouth County | " | 9-15-73<br>9-16-73 | Brain-EE | Negative | B1 1280<br>B2 640 | 64<br>32 | ND | Positive |
| Cream Ridge<br>Monmouth County | " | 9-23-73 | Brain-EE | Negative | B1<br>160 | Unsat. | ND | Positive |
| Milford<br>Delaware * | " | 9-24-73 | Brain-EE | No blood<br>Submitted | --- | --- | --- | Positive |
| Salem<br>Salem County | " | 9-28-73 | No Brain<br>Submitted | Negative | B1<br>2560 | ND | ND | No Brain<br>Submitted |
| Blue Anchor<br>Camden County | " | 10-1-73 | No Brain<br>Submitted | Negative | B1<br>160 | Unsat. | ND | No Brain<br>Submitted |
| Salem<br>Salem County | " | 10-9-73<br>10-31-73 | No Brain<br>Submitted | Negative | B1 2560<br>B2 1280 | 128<br>128 | ND<br>ND | No Brain<br>Submitted |

* Included are two horses from the State of Delaware

| Group | Virus and No. of Strains | | Isolated from | Collected in | Month | Year |
|---|---|---|---|---|---|---|
| | EE | WE | | | | |
| A | 6 | | C.melanura | Bass River | July | 1973 |
| A | 8 | | C.melanura | Woodbine | July | 1973 |
| A | 1 | | C.melanura | Ocean County | July | 1973 |
| A | 5 | | C.melanura | Bass River | August | 1973 |
| A | 1 | | C.melanura | Cumberland Co. | August | 1973 |
| A | 4 | | C.melanura | Woodbine | August | 1973 |
| A | 1 | | A.crucians | Woodbine | August | 1973 |
| A | 8 | 1 | C.melanura | Ocean County | August | 1973 |
| A | 1 | | C.salinarius | Ocean County | August | 1973 |
| A | 4 | | C.melanura | Route 9 | August | 1973 |
| A | 2 | | C.melanura | Woodbine | September | 1973 |
| A | 3 | 1 | C.melanura | Woodbine | October | 1973 |
| Totals | 44 | 2 | | | | |

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY
CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, NEW YORK

Lethalities and virulences of Venezuelan encephalitis viruses for hamsters correlated with severity of histopathologic lesions in hematopoietic and brain tissues. Virulent subtypes I and II strains produced lesions in both tissues whereas virulent subtype III strains usually caused no lesions in hematopoietic tissues, and deaths were related chiefly to hemorrhagic brain lesions and necrosis of neuronal cells. Two hamster-benign viruses (the TC83 attenuated vaccine strain and subtype IV) usually caused no lesions. Leucopenia occurred in hamsters infected with either virulent or benign strains of VE virus, but disappeared only in benign virus infections. The mechanism was probably a redistribution of leucocytes in the body rather than their destruction. Growth curves of virulent and benign VE viruses in hamsters as reflected by virus concentrations in blood, bone marrow, lymphoid tissues and brain did not correlate with abilities of the viruses to kill hamsters when given subcutaneously. Even intracranial or intracardiac inoculation did not increase lethalities of benign strains to those of virulent viruses. VE virus strains from French Guiana and clones from virulent strains are under examination in search for additional hamster-benign VE viruses to study. Baseline methodologic experiments indicated that polyethylene glycol purification was effective for VE virus provided sonication was utilized presumably to detach virus from the polyethylene glycol precipitate. VE viruses were found to multiply in hamster peritoneal cells including macrophages, but both hamster-virulent and hamster-benign strains caused cytopathic effects. Although cells in hematopoietic tissues of hamsters are destroyed by virulent VE viruses and essentially all cells contain viral antigen as demonstrated with fluorescent-antibody staining, infectious center assays of cells from bone marrow and Peyer's patches have failed to show over 10% of cells producing infectious virus. Steps in the technic of the infectious center assay did not explain this discrepancy between infectious center results and fluorescent antibody data. The correlation between plaque size of Central American epizootic and enzootic VE viruses in vero African green monkey kidney cell cultures as originally reported by the Middle America Research Unit, did not extend to LLCMK2 rhesus monkey kidney nor to primary chicken embryonic cell cultures.

In a collaborative project with Walter Reed Army Institute of Research, Dr. P. Russell, the Pacific Research Section of the National Institute of Allergy and Infectious Diseases, Dr. L. Rosen, and Yale University, Dr. J. Casals, chimpanzees were inoculated with single strains of each of the four serotypes of dengue viruses. The strains were obtained from sick humans and used at low passage levels from isolation. Each chimpanzee produced broad dengue and group B arbovirus antibody responses by HI test, but no clinical illness nor subclinical changes in leucocytes, platelets or hematocrits could be detected. Three chimpanzees were young animals born in the United States. Viremia patterns are being determined.

Studies of arboviruses from the Amazon region of Peru on the eastern side of the Andes mountains during 1970-1971 have yielded strains of VE, eastern encephalitis, several types of group C, probably more than one type of Guama and a virus not in groups A, B or C. Some strains of Peruvian VE viruses have plaque sizes in vero cell cultures that were as large as subtype I, variety E, but some strains produced smaller plaques in between the sizes of varieties B and E of subtype I. One VE virus strain, 71D1252, may

represent a new subtype since it was clearly a VE virus by CF test, but was not neutralized by small amounts of antibody to any of the four currently known subtypes. The eastern encephalitis viruses found in the Amazon region of Peru may be relatively benign for equines since horses in that area had antibody without histories of encephalitic illness.

Search for persistent epizootic-epidemic VE virus in Central America was expanded in 1972 by studies with sentinel horses, sentinel hamsters, mosquitoes and wild mammals in southeastern Pacific coastal Guatemala at the epicenter of the 1969 outbreak, in an adjacent region of El Salvador and in Pacific coastal Nicaragua where horse deaths presumably due to encephalitis occurred during April-June 1972. To date in the epicenter area on the Pacific coast of Guatemala, sentinel horses have not developed VE virus antibody, hamsters have not died and wild terrestrial mammals were without antibody in 1972. Across the border in El Salvador, horses born since the 1969 outbreak were without detectable HI or neutralizing antibody to VE virus and humans had antibody in a prevalence and with age and sex distributions compatible with a single sweep of virus through the area in 1969. However in Nicaragua, some young horses born since the 1969 outbreak had neutralizing antibody in serum that was higher in titer against an epizootic virus strain than versus an enzootic strain, suggesting persistence of the epizootic virus in that region. Investigations of possible ebb and flow movements of VE virus from an enzootic focus at La Avellana on the Pacific coast of Guatemala utilizing sentinel horses and hamsters have demonstrated a gradient of virus radially from the marsh and virus activity in insular wooded habitats during one wet but not one dry season. During 1970-1971 about 10% of resident birds and bats at La Avellana were infected by VE virus whereas over 70 percent of terrestrial mammals were infected including humans, dogs, small rodents and opossums. Rabbits and chickens were poor sentinels for VE virus at La Avellana in contrast to hamsters. One of three strains of Capim group arboviruses isolated from sentinel hamsters exposed 13 kilometers north of La Avellana is related to a Capim group virus from Brazil (BeAn84381), but the two other strains are different and are neither BeAn84381 nor Acara virus although all three react by complement-fixation with Acara antiserum. Three additional strains of an unidentified virus (70U39) were isolated from sentinel hamsters on the Pacific coast of Guatemala during 1972 at a marsh 9 kilometers from the site of original isolation in 1970.

Human sera from Louisiana collected during the period 1955-69 were without detectable N or HI antibodies to VE viruses. Spiny rats from Trinidad often had pre-existing antibody to VE virus and thus were not useful for production of antibodies although small quantities of ascitic fluid could be produced by intraperitoneal inoculation of complete Freund's adjuvant. A Florida strain of VE virus, Fe5-47et, produced antibodies in rabbits which broadly neutralized subtypes I, III and IV viruses. A short febrile illness caused by a group C arbovirus occurred in a person working in the field during August 1972 on the southeastern Pacific coast of Guatemala.

(William F. Scherer)

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

Report for January - December 1973

During 1973, 27 strains of virus were isolated, 25 from mosquitoes and 1 each from a bird and horse. Approximately 100,000 arthropods were processed in 2,592 pools, and 107 birds and 59 non-avian vertebrates were examined for virus. HI tests were done on the sera of 1,744 birds, 583 other vertebrates, and 106 apparently healthy humans as well as of 349 patients with suspected CNS infections.

California encephalitis

Three cases of CE were serologically confirmed during 1973. The patients were 8 to 12 years of age and resided in Albany County. The onset of illness was in July, August and September. Antibody rises were observed in 1 case by N test, in 2 cases by CF test and in all cases by HI test. All patients had N antibody for the New York State strain of Snowshoe Hare virus. A CSF specimen collected from 1 patient on the fourth day of illness did not yield virus.

In 3 additional children, age 3 years or less and residing in the same area, a presumptive diagnosis of CE was made, based on findings of HI and N antibody in single serum specimens. Five apparently healthy family members or neighbors of the Albany area patients had HI and/or N antibody.

A single specimen from an 11-year-old child from Washington County had a N index of 3.1 to the New York strain of Snowshoe Hare virus but showed no protection against the LaCrosse strain.

Three isolations of CE group viruses were made from *Aedes* mosquitoes (Tables 1 and 2), none of which were collected in the area of the human cases. The isolation from *Aedes cantator* is the first record of a CE group virus from this species.

Arbovirus activity in Suffolk County

Suffolk County was the site of intense arbovirus activity during the summer of 1973. EE, WE, CE, Flanders (FLA) and 1 unidentified virus were isolated from mosquitoes, and 1 bird yielded WE virus (Tables 1 and 2). The WE isolates are the first reported of this virus from New York State.

EE and WE viruses from mosquitoes and birds. One isolation of EE virus was obtained from a pool of 100/3,904 *Culex pipiens* mosquitoes taken in August.

Seven isolations of WE virus were made from 8 pools of 100 Culiseta melanura caught in the last week of August within a one-mile radius of a small brackish swamp at Montauk. Two pools totaling 170 mosquitoes captured 2 weeks earlier at the same locality were negative.

One isolation of WE virus was made from a brain and liver pool obtained from 1 of 4 yellowthroats tested. This bird and 9 others, all captured in the month of August, were negative for EE and WE HI antibodies.

A total of 284 wild birds of over 20 species captured from June through September in Suffolk County was examined for HI antibodies to EE and WE virus. The number of species found to have antibody rose from 5 in the period June through August to 15 in September. Of 9 species which were antibody positive in September and also captured in previous months, only 2 had antibody before September. The antibody rate for the birds of these 9 species rose from 3% (of 150) to 60% (of 40) for EE, and from less than 1% to 35% of the same totals for WE. There were no sentinel pheasant conversions for EE virus, but 3 of these birds developed antibodies to WE virus by August.

Strain variations of WE virus. None of the WE virus isolates reacted in CF test with mouse sera prepared against the McMillan strain of WE virus. They were identified by N test with WE strain A-42 isolated from a horse in California. Of 48 birds tested by HI with McM and by N test with A-42, only 44% were positive by HI as compared with 69% by N test. This lack of correlation could be due to strain variation. Karabatsos et al. have previously shown that local strains of WE virus detect higher HI antibody rates in East-Coast birds than do the prototype strains. The correlation between HI and N test results in 50 birds examined for EE antibody was 96%.

Epidemiology. In spite of the marked increase in EE antibody in wild birds towards the end of the summer, Culiseta melanura, the enzootic vector of EE, was surprisingly free of detectable EE virus. Large numbers of epidemic vectors (Aedes) processed in August were negative for virus and there was apparently no spread to pheasants or horses. This contrasted with the situation in New Hampshire, Massachusetts, Rhode Island and New Jersey where large outbreaks in horses and pheasants, as well as a number of human infections occurred.

The high incidence of WE infection in C. melanura from a single breeding site, and the isolation of WE from a bird caught nearby, suggests the existence of a geographically restricted focus of infection in Montauk. Birds have been observed flying between here and New England where many isolations of WE virus were made this summer.

CE virus from mosquitoes. A pool of 50/1,908 *Aedes cantator* captured in June yielded CE virus. Although comparable numbers of this species were tested in July and August, no further isolations were obtained. As in 1972, CE-infected mosquitoes appeared earlier in the year than mosquitoes infected with any other arbovirus.

Flanders virus from mosquitoes. Thirteen isolations of this avian rhabdovirus were made from *Culex* and *Culiseta* mosquitoes collected in July and August.

(John P. Woodall and Newton Perrins)

Table 1

Arbovirus Isolations from Arthropods, Wildlife and Domestic Animals, 1973

| Virus | No. of Strains | County | Month Collected | Species |
|---|---|---|---|---|
| CE | 1 | Suffolk | June | *Aedes cantator* |
| CE | 1 | Warren | July | *Aedes stimulans* |
| CE | 1 | Cattaraugus | July | *Aedes stimulans* |
| EE | 1 | Suffolk | August | *Culex pipiens* |
| EE | 1 | Onondaga | October | Horse |
| WE | 1 | Suffolk | August | Yellowthroat |
| WE | 7 | Suffolk | August | *Culiseta melanura* |
| Flanders | 8 | Suffolk | July | *Culex pipiens* |
| Flanders | 2 | Suffolk | July | *Culex salinarius* |
| Flanders | 2 | Suffolk | August | *Culex pipiens/restuans* |
| Flanders | 1 | Suffolk | August | *Culiseta melanura* |
| Unidentified | 1 | Suffolk | August | *Aedes cantator* |

Table 2

Mosquitoes Examined for Arboviruses in Suffolk County, June-September 1973*

| Species | June | July | August | September |
|---|---|---|---|---|
| *Aedes canadensis* | 1,085 | 454 | 498 | |
| *Aedes cantator* | 1,908 (CE 1) | 1,457 | 2,573 (unk. 1) | |
| *Aedes sollicitans* | 69 | 1,862 | 8,219 | |
| *Aedes vexans* | 188 | 162 | 3,732 | |
| *Anopheles punctipennis* | | | | 180 |
| *Culex pipiens* | 22 | 5,221 (FLA 8) | 3,904 (EE 1) | 229 |
| *Culex pipiens/restuans* | 94 | 814 | 5,900 (FLA 2) | |
| *Culex salinarius* | 382 | 3,815 (FLA 2) | 695 | |
| *Culiseta melanura* | | 47 | 1,452 (FLA 1) (WE 7) | 450 |
| *Mansonia perturbans* | 534 | 5,068 | 300 | |

*Type and number of virus strains isolated are shown in parenthesis.

REPORT FROM THE UNIVERSITY OF WISCONSIN
DEPARTMENTS OF PREVENTIVE MEDICINE, ENTOMOLOGY AND
VETERINARY SCIENCE, MADISON, WISCONSIN

Twenty-four cases of California encephalitis (LaCrosse) were serologically diagnosed in Wisconsin in the 1973 season. The age and sex distribution were: 13 boys and 11 girls, three months to 17 years of age. Fifteen were residents of Wisconsin, 4 from southeastern Minnesota, and 5 from northeastern Iowa. Five cases had dates of onset in July, 10 during August and 9 in September. The distribution of these cases is similar to that of 165 cases diagnosed during pervious years in Wisconsin.

The continuing appearance of this virus, season after season, in the hardwood deciduous forests as found in the southwestern third of Wisconsin, led to further studies as to the natural cycle of the virus, and how it might survive in the area.

The isolation of LAC virus from mosquito larvae, in June of 1972, was confirmed during 1973. Overwintering of LAC virus in *Aedes triseriatus* larvae was demonstrated by additional isolations from larvae collected in the field before the seasonal emergence of adults. Larger sampling of eggs and larvae were collected from tree-holes in the same and several additional known endemic areas early in the spring of 1973, and tested for virus. Larvae not tested were reared to adults, some of which were assayed only for virus while others were allowed to feed on mice and then sacrificed for infectivity assay. The number of male mosquitoes per pool ranged from 1 to 12 and the number of females per pool ranged from 1 to 9 mosquitoes.

LAC virus was isolated from 3 pools of *A. triseriatus* larvae and from 1 pool of adult male mosquitoes reared from larvae collected from basal tree-holes during March, April, May and June of 1973. Isolations of this virus were also obtained from 5 suspensions of *A. triseriatus* larvae and 4 suspensions of adult male mosquitoes originating from old automobile tires (Table 1). Virus transmission to suckling mice was accomplished by female *A. triseriatus* reared from larvae collected from the tires (Table 2). Only two tree-holes and the tires yielded LAC virus infected larvae and adults (Tables 3 and 4). No virus was isolated from 255 female mosquitoes reared from larvae collected from the tree-holes. These findings strongly imply that *A. triseriatus* is the reservoir of LAC virus and that transovarial transmission of the virus in *A. triseriatus* is the mechanism responsible for the survival of this arbovirus during the winter season in the North Central United States.

Assessment of relative vector potential of *A. hendersoni*, compared to *A. triseriatus*, is underway. These species were fed different concentrations of LAC virus in defibrinated blood through a lamb-skin membrane. Infection and transmission rates for these species following 14 days or more incubation are presented in Table 5. Each mosquito species was readily infected as indicated by an 87% rate for *A. triseriatus* and a 75% rate for *A. hendersoni*. The overall transmission rate for *A. triseriatus* was 61% while *A. hendersoni* failed to transmit a lethal dose of virus to suckling mice.

Infection and transmission rates for hybrids of A. triseriatus X A. hendersoni are also being studied. Preliminary results indicate that hybrids are intermediate between A. triseriatus (high) and A. hendersoni (no transmission) in their ability to transmit LAC virus. These studies are being expanded to compare hybrids and parent stock A. triseriatus and A. hendersoni from throughout Wisconsin, including areas outside of the LAC endemic area.

The role of different virus subpopulations in the endemic maintenance cycles of California group viruses is being studied. LAC virus prototype produces both large and small plaques in Vero cells. The role of these different plaque types in human disease and in the natural endemic cycle is presently being investigated. Large and small plaque type LAC virus was purified by plaque selection in terminal dilution. Passage of prototype LAC virus in chipmunks produces predominantly large plaque progeny virus. LAC virus recovered from naturally infected A. triseriatus adults and larvae is also comprised predominantly of large plaques. The prototype LAC virus passaged in gray squirrels, however, produces predominantly small plaque virus. The selective virulence of these plaque types in mice, possible antigenic differences and their infection and transmission rates in A. triseriatus is under study.

Evaluation of serological data from our collaborative arbovirus studies with the Veterinary faculty, Universidad of Antioquia, is in progress. VSV-Indiana and New Jersey appeared to be prevalent in all four altitudinal zones studied, including the remote forested site. Seasonal increases in antibody prevalence on wild vertebrates appears to correspond to onset of clinical cases in livestock, generally. Wild vertebrates resident in clearings appeared to have had higher antibody prevalence rates than those from forests. A virus isolated from a pool of engorged Simulium exiguum has been identified as a strain of Venezuelan equine encephalitis. Relatively low antibody prevalence rates were detected in wild mammals, and lower rates were observed among wild birds.

(W.H. Thompson, G.R. DeFoliart, T.M. Yuill, D.M. Watts, P.R. Grimstad, W.R. Hansen, B.D. Nassif, F.N. Zuluaga, B. Beaty)

Collected during the Spring of 1973.*

| Breeding Sites | | April 20-26 | May 10-17 | 23-31 | June 7 | Total |
|---|---|---|---|---|---|---|
| PF-11 | tree-hole | 1/7(140)** | 0/5(50) | --*** | -- | 1/12(190) |
| PF-12 | " | 0/10(200) | -- | -- | -- | 0/10(200) |
| DF-13 | " | 0/16(160) | 0/7(70) | -- | -- | 0/23(230) |
| DF-14 | " | 0/7(70) | 0/3(30) | 0/1(14) | -- | 0/11(114) |
| GB-02 | " | 0/13(140) | 0/2(20) | 0/1(10) | -- | 0/16(170) |
| GB-03 | " | 0/15(150) | 0/1(10) | 0/2(20) | -- | 0/18(180) |
| FD-08 | " | 0/4(28) | 0/7(70) | -- | -- | 0/11(98) |
| GW-15 | " | 0/2(20) | 1/3(30)** | 1/7(70)** | 0/2(20) | 2/14(140) |
| SRC tires | | -- | -- | 5/10(100)** | -- | 5/10(100) |
| TOTAL | | 1/74(908) | 1/28(280) | 6/21(214) | 0/2(20) | 8/125(1422) |

* Larvae collected during March were allowed to develop to adults.
** Pools yielding LAC virus/number pools tested (total number individual larvae).
*** Larvae either not collected or allowed to develop to adults.

Table 2 LAC Virus Isolations from Adult *A. triseriatus* Originating from Field Collected Larvae, and Transmission of Virus to Mice

| Breeding Sites | +Pools/Pools (No. Individuals) | | No. Trans./No. Fed | Total* | |
|---|---|---|---|---|---|
| | Males | Females | Females | Females | Males and Females |
| PF-11 tree-hole | 1/09(41)** | 0/8(17) | 0/33*** | 0/20(50) | 1/29(91) |
| GW-15 tree-hole | 0/20(110) | 0/4(11) | 0/40 | 0/21(51) | 0/41(161) |
| Six Other Sites | 0/32(159) | 0/18(62) | 0/92 | 0/58(154) | 0/90(313) |
| SRC tires | 4/11(108)** | 0/8(34) | 1 or 2/76** | 1/29(110) | 4/40(218) |
| TOTAL | 5/72(418) | 0/38(124) | 1 or 2/241 | 1/128(365) | 5/200(783) |

* Total number pools yielding LAC virus/total number pools tested (total number individuals per breeding site).

** Pools yielding LAC virus/number pools tested (total number individual mosquitoes).

*** Number individual(s) mosquitoes transmitting virus/number individuals feeding on mice.

Table 3 Summary of LAC Virus Isolations Obtained from *A. triseriatus* Larvae or Adults

| Breeding Site | Collection Date | Isolate Pool No. | Isolations Larvae | Isolations Male | Isolations Female |
|---|---|---|---|---|---|
| PF-11 | 4/26/73 | 27* | 1/20** | | |
| PF-11 | 5/17/73 | 231* | | 1/10 | |
| GW-15 | 5/17/73 | 125 | 1/10 | | |
| GW-15 | 5/23/73 | 166* | 1/10 | | |
| SRC tires | 5/23/73 | 132 | 1/10 | | |
| SRC tires | 5/23/73 | 133 | 1/10 | | |
| SRC tires | 5/23/73 | 134 | 1/10 | | |
| SRC tires | 5/23/73 | 135* | 1/10 | | |
| SRC tires | 5/23/73 | 138 | 1/10 | | |
| SRC tires | 5/23/73 | 202* | | 1/10 | |
| SRC tires | 5/23/73 | 203* | | 1/10 | |
| SRC tires | 5/23/73 | 206* | | 1/11 | |
| SRC tires | 5/23/73 | 316* | | 1/5 | |
| SRC tires | 5/23/73 | 341* | | | 1/2* |

*Reisolation of virus from original mosquito or larvae suspension

**One pool/number per pool

Table 4 Identification of Isolates Based on Differential Neutralization of Infectivity by La Crosse (LAC), Snowshoe Hare (SSH) and Jamestown Canyon (JC) Hyperimmune Fluids.

| Hyper-immune Fluids* | Viruses | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LAC | SSH | JC | PF L-27 | PF M-231 | GW L-125 | GW L-166 | SRC L-134 | SRC L-138 | SRC M-202 | SRC M-316 | SRC F-341 | SRC 341T** |
| LAC | 3.8*** | 2.5 | 2.2 | 3.8 | 3.7 | 3.5 | 4.0 | 3.8 | 4.3 | 4.6 | 3.7 | 4.2 | 3.5 |
| SSH | 2.3 | 3.5 | 2.0 | 2.5 | 2.4 | 2.3 | 2.4 | 2.8 | 3.3 | 3.3 | 2.2 | 3.2 | 2.8 |
| JC | 2.0 | 1.2 | 3.2 | 0.8 | 1.1 | 0.7 | 1.2 | 1.0 | 1.3 | 0.8 | 0.2 | 1.0 | 1.2 |

*Hyperimmune fluid employed at 1:80 dilution

**Brain suspension of mouse that died after being fed on by 2 mosquitoes.

***$Log_{10}$ neutralization index

L--isolate from larvae; M--isolate from adult male; F--isolate from adult female.

Table 5. Infection and transmission rates for *Aedes triseriatus* and for *Aedes* [illegible] different concentrations of LaCrosse virus in defibrinated blood and through feeding on viremic chipminks.

| Virus dose | Extrinsic incubation (days) | | Number of mosquitoes | | Infection rates (%) | | Transmission rates (%) | |
|---|---|---|---|---|---|---|---|---|
| | A. tris. | A. hend. | A. tris. | A. hend. | A. tris. | A. hend. | A. tris. | A. hend. |
| 5.0-6.0 | 21 | 21 | 8 | 12 | 88 (7/8) | 92 (11/12) | 80 (4/5) | 0 (0/6) |
| 5.0 | 0 | 7,14,21,27 | 0 | 16 | 0 | 75 (12/16) | 0 | 0 (0/16) |
| 4.5 | 24 | 24 | 5 | 5 | 100 (5/5) | 80 (4/5) | 100 (5/5) | 0 (0/5) |
| 3.5* | 14 | 14,21 | 5 | 12 | NT† | 75 (9/12) | 40 (2/5) | 0 (0/12) |
| 3.4* | 15,17 | 14,21 | 7 | 7 | 100 (7/7) | 43 (3/7) | 57 (4/7) | 0 (0/7) |
| 4.7 | 16,20 | 16,20 | 18 | 11 | NT† | NT† | 56 (10/18) | 0 (0/11) |
| 6.2 | 16,18 | 16,18,19 | 6 | 19 | 67 (4/6) | 75 (9/12) | 50 (3/6) | 0 (0/19) |
| Totals | | | 49 | 82 | 87 (23/26) | 75 (48/64) | 61 (28/46) | 0 (0/76) |

* Fed on virenic chipmunks

† [illegible]

## Cultivation of high infectivity of Japanese Encephalitis virus in BHK-21 shaker cultures employing reduced amounts of serum in Leafhopper medium.

High titers of Japanese encephalitis virus (JEV) were obtained in BHK-21 cells adapted to grow in shaker culture using reduced amounts of calf serum (2.5%) and medium supplemented with lipids. Titers ranging between $10^8$ and $10^9$ plaque forming units (PFU) per ml were obtained by reducing the volume of the medium 12 hours after infection to 10% or 25% of the original volumes, depending on whether cells were cultivated in shaker cultures or roller tubes.

Comparable titers were not reached when infected cultures were cultivated in higher amounts of calf serum. This reduced capacity to obtain high titers of JEV may be due to either a detrimental effect of the calf serum on the cells or to a nutritional based structural change in cells resulting in reduced adsorption or attachment of JEV. Further studies of this observation are in progress. (Dr. Louis Guskey, K. Crilly and Howard M. Jenkin).

## Growth of Japanese encephalitis virus in Aedes aegypti (AE) spinner cultures and stability of the released virus.

The release of JEV from infected AE cells (Singh) was studied during a 72-hr. incubation period. The infected cells were cultivated in suspension culture at 28 C in Leafhopper medium containing 2% newborn calf serum. Infectivity of JEV was measured as plaque-forming units (PFU) per ml in monolayers of BHK-21 cells. There was a 5 log increase of infectivity in the supernatant fluid after a 36-hr. incubation period. At peak infectivity, the titer was $8 \times 10^7$ PFU per ml. No difference in infectivity of virus was observed after titrating freshly harvested virus immediately or frozen virus held for 1 month at -80 C in spent medium. There was a 90% reduction of infectivity when the virus was incubated for 96 hr. in cell-free fresh Leafhopper medium at 28 C. A 40-50% reduction in infectivity occurred during the same incubation period when virus was incubated in cell-free spent medium obtained 24, 48, and 72 hr. after infection. The high yield of JEV from infected AE cultivated in low serum-containing medium can prove to be a good source of virus for antigen. (Lloyd E. Anderson and Howard M. Jenkin)

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

Arbovirus activity in man: Arbovirus activity in South Dakota was examined by testing human serum samples collected from Custer State Hospital (CSH) residents October 19, 1972. The 177 residents tested were persons from all areas of South Dakota who were institutionalized at Custer because of severe mental retardation or physical deformities requiring constant hospital supervision. Of the 177 Custer patients tested for HAI antibodies to Western Equine Encephalitis (WEE) and St. Louis Encephalitis (SLE), 32 were found positive for SLE and 2 positive for WEE (HAI titer 40 or greater). Detailed Custer State Hospital patient histories are shown in table 1. It may be noted from the table that over ninty percent of the positive Custer patients are within an age group of 0-30 years. Since patients at the Custer Hospital are confined to bed because of severe deformities, the arbovirus antibodies detected should reflect virus exposure levels prior to hospital admittance. Based on this assumption, the statewide distribution of positive patients was determined and is mapped in Figure 1. It is interesting to note from the map that 19 of the 33 positive patients are from two areas of eastern South Dakota. An index of infection rate per 1,000 persons of the age group 0-30 is given in Table 1. To help determine a statewide peak virus exposure period (epidemic period during last decade), the years of patient residence at Custer was examined. Figure 2 shows the positive patient level compared with years of residence at CSH. Evidence in Figure 2 suggests that the last peak arbovirus exposure period in South Dakota occurred between the years 1963 and 1966.

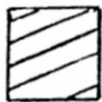
Virus isolation from mosquitoes: Mosquitoes trapped at four major sites in South Dakota (Brookings, La Creek National Wildlife Refuge, Angostura Irrigation District and Redfield) in the summer seasons of 1969 through 1972 have yielded 78 confirmed arbovirus isolates. Detailed information showing monthly distribution of all virus isolations from South Dakota mosquitoes is given in Table 2. It may be noted that of the 78 total viruses, 49 were isolated from Culex tarsalis mosquitoes collected during the month of August. Also observable from the table, over fifty percent of the 49 August isolates were WEE. This monthly distribution of WEE corresponds directly with reported equine encephalitis cases in South Dakota.

Arbovirus activity in migratory waterfowl: In 1973 serum samples were obtained from 105 total Canada Geese and cormorants at Waubay National Wildlife Refuge, located in northeastern South Dakota. The samples were tested for HAI antibodies to SLE, WEE, and Venezuelan Equine Encephalitis (VEE). Over 25 percent of all 1973 waterfowl samples were found positive for WEE antibody. A smaller antibody percentage level was detected for SLE, 2.4% in Canada geese and 12.7% in cormorants. VEE tests performed revealed no positives for either bird population. Comparative results for waterfowl studies from 1970 through 1973 are given in Table 3. The three-year data presented in Table 3 provides an indicator of how WEE and SLE may be picked up and amplified in migratory bird populations. The presented data suggests that SLE antibody levels in Canada geese increase from a south to north direction through the Dakotas. WEE data shows a less ideal pattern, perhaps indicating that peak WEE exposure occurs before the migratory birds reach the Dakotas.

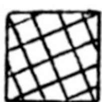
(G. C. Parikh, C. D. Wilson, R. F. Schryer)

... and/or WEE positive Custer State Hospital patients.

Missouri River



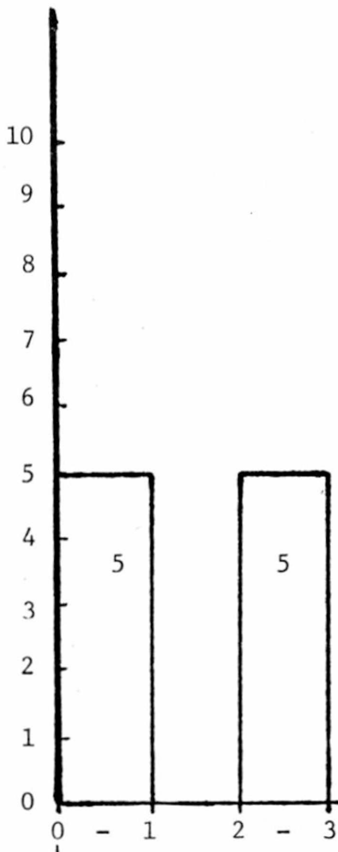
\- Lake Areas (Pothole ecology area)



\- River Lowland Areas (James River Valley)

represents one Custer State Hospital patient positive for SLE

Number of
Custer
Patients
Positive
for WEE
and/or SLE



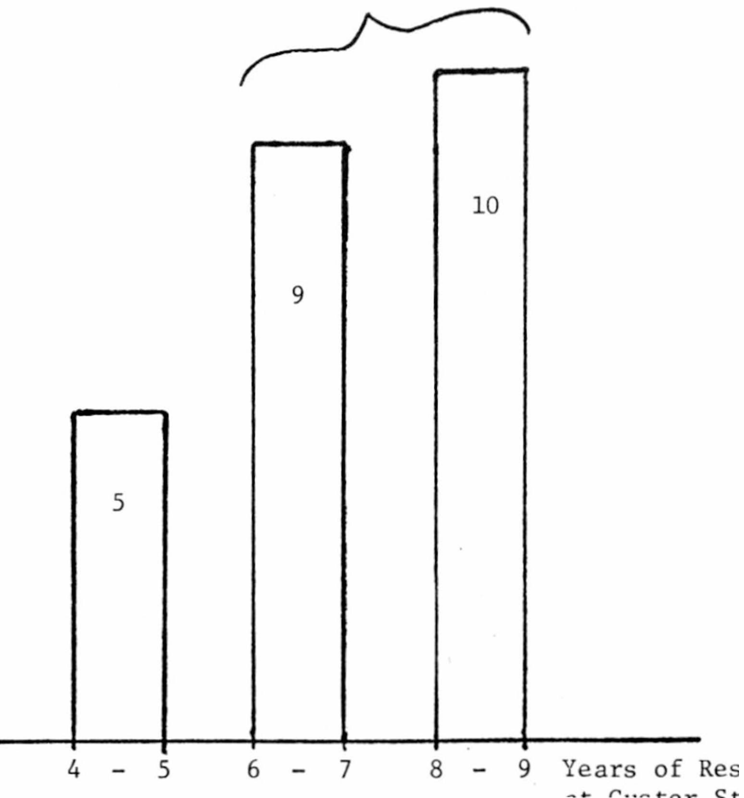

Peak Period Observed
5
9
10
4 - 5
6 - 7
8 - 9
Years of Res

Table 1. Custer State Hospital (CSH) patients testing positive ($\geq$ 40 HAI titer) for WEE or SLE antibodies, October 19, 1972.

| County Residence (Pre CSH) | Town | Age | Sex | Clinical Diagnosis | Years of Residence (at CSH) | Arbovirus Antibody Detected | 1970 Census Data - Age Group 0-30 | Infection Rate/1000 Persons Age 0-30 |
|---|---|---|---|---|---|---|---|---|
| Beadle | Wolsey | 12 | F | MR[1], Undifferentiated | 5 | SLE | 10,585 | .0945 |
| Brown | Aberdeen | 17 | M | MD[2], familial | 2 | WEE | 20,747 | .0482 |
| Campbell | Artas | 21 | F | MD[2], Post-infectional | 9 | SLE | 1,448 | .6906 |
| Charles Mix | Lake Andes | 7 | F | Clinical variety of MD[2] | 2 | SLE | 5,008 | .1997 |
| Clark | Vienna | 16 | M | Severe MR[1], congenital | 7 | SLE | 2,317 | .4316 |
| Codington | Watertown | 9 | M | MR[1], severe, probably post-traumatic | 5 | SLE | 9,698 | .1031 |
| Corson | McLaughlin | 39 | F | MD[2], severe, with epilepsy | 5 | SLE | 2,925 | .3419[3] |
| Custer | Custer | 19 | M | MR[1], profound, due to traumatic cerebral hemorrhage at age 3 mo. | 3 | SLE | 2,279 | .4388 |
| Davison | Mitchell | 10 | M | MR[1], with cerebral palsy of athetoid quadraplegic type | .08 | SLE | 8,925 | .112 |
| Grant | Milbank | 9 | F | MR[1], with hydrocephalus | 2 | SLE | 4,421 | .2262 |
| Gregory | Bonesteel | 22 | F | MD[2], with cerebral spastic infantile paralysis | 6 | SLE & WEE | 3,093 | .6466 |
| Hamlin | Lake Norden | 36 | F | MR[1], profound, probably congenital brain defect | 4 | SLE | 2,288 | .4371[3] |
| Hand | Miller | 18 | M | Cerebral palsy, epileptic | 8 | SLE | 2,933 | .3409 |
| Hutchinson | Freeman | 12 | M | MR[1], moderate, undifferentiated | 2 | SLE | 4,623 | .4326 |
| Hutchinson | Tripp | 15 | M | MD[2], with cerebral spinal infantile paralysis | 9 | SLE | | |
| Kingsbury | De Smet | 11 | M | MR[1], severe, spastic paralysis, hydrocephalus, retina blastoma | 7 | SLE | 3,361 | .5951 |
| Kingsbury | Erwin | 19 | M | MD[2] with developmental cranial anomalies | 9 | SLE | | |
| Lake | Madison | 10 | M | MR[1], severe, hydrocephalus | 7 | SLE | 5,950 | .3361 |
| Lake | Madison | 28 | M | MR[1], severe, mongoloid | 9 | SLE | | |
| Lawrence | Spearfish | 25 | F | MD[2] with congenital spastic infantile paralysis, Mongoloid | 9 | SLE | 9,664 | .1035 |
| Mellette | Norris | 8 | M | MR[1], organic brain syndrome of unknown etiology | 1 | SLE | 1,379 | .7252 |
| Minnehaha | Sioux Falls | 9 | M | MR[1], severe, brain damage | 7 | SLE | 52,504 | .0381 |
| Minnehaha | Sioux Falls | 15 | F | MD[2] due to Agenesis | 9 | SLE | | |
| Pennington | Owanka | 25 | F | MD[2], post infectional (Encephalitis) | 9 | SLE | | |
| Pennington | Rapid City | 10 | F | MR[1], severe with epileptic seizures, possible birth trauma and anoxia | 7 | SLE | 34,757 | .1439 |
| Pennington | Rapid City | 18 | F | MR[1], cerebral palsy | 7 | SLE | | |
| Pennington | Rapid City | 21 | F | MD[2] | 5 | SLE | | |
| Pennington | Rapid City | 23 | M | MD[2] with developmental cranial anomalies | 9 | SLE | | |
| Roberts | Sisseton | 7 | M | MR[1], severe, undifferentiated, Microcephalic | 3 | SLE | 5,745 | .1741 |
| Shannon | Pine Ridge | 15 | M | MR[1], undifferentiated, probably congenital | 9 | SLE | 5,476 | .1826 |
| Tripp | Winner | 42 | F | MR[1], profound with epilepsy, Post car accident | 1 | SLE | 4,121 | .2427[3] |
| Yankton | Yankton | 9 | F | MR[1] with Epilepsy | 1 | SLE | 9,763 | .1024 |
| Ziebach | Cherry Creek | 10 | M | MR[1], severe | 7 | SLE | 1,368 | .7310 |

[1]MR - Mental Retardation

[2]MD - Mental Deficiency

[3]Infection rate was determined as if patient were within age group 0-30.

Table 2. Monthly distribution of arboviruses isolated from mosquitoes collected in South Dakota, 1969-1972.

| Month of isolation | Virus Strain | | | | | |
|---|---|---|---|---|---|---|
| | WEE | SLE | FLA | CAL | CVV | TUR |
| June | 1 | | 2 | | | |
| July | 8 | 2 | 6 | 5 | 2 | |
| August | 25 | 14 | 5 | 1 | 3 | 1 |
| September | 2 | 1 | | | | |
| Total | 36 | 17 | 13 | 6 | 5 | 1 |

Table 3. Indication of participatory role in encephalitis of migratory waterfowl nesting in Dakotas.

| Location | Year | Waterfowl | Sample Size | Percentage of birds with antibody | |
|---|---|---|---|---|---|
| | | | | WEE | SLE |
| Martin - La Creek National Wildlife Refuge (Western South Dakota) | 1970 | Mallard, blue-wing teal | 63 | 3.2 | 0.0 |
| | 1970 | Canada Geese* | 50 | 22.0 | 0.0 |
| | 1971 | Canada Geese* | 56 | 12.5 | 5.4 |
| Waubay - Waubay National Wildlife Refuge (Eastern South Dakota) | 1971 | Canada Geese | 180 | 23.3 | 6.6 |
| | 1972 | Canada Geese | 97 | 8.2 | 4.1 |
| | 1973 | Canada Geese | 42 | 26.2 | 2.4 |
| | 1973 | Cormorant | 63 | 25.4 | 12.7 |
| Jamestown, ND - Northern Prairie Waterfowl Research Center | 1972 | Canada Geese* | 100 | 2.0 | 22.0 |
| Total | | | 651 | 15.2 | 7.7 |

*These Canada Geese were captive throughout the year.

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

I. Ecology of Western Encephalitis (WE) Virus in the United States.

A. Morgan County, Colorado.

The finding of unexpectedly high infection rates for western encephalitis (WE) virus among mosquitoes and flocks of chickens along the South Platte River drainage in eastern Colorado during 1973 led to additional studies in Morgan County, Colorado during 1973. The follow-up studies were conducted to determine if the high levels of mosquito infections and chicken flock serologic conversions reoccurred in the area. Studies of nestling birds also were made during the period from June 27 through September 12, 1973, to determine if nestlings were involved in the WE virus summer amplification cycle in Colorado as house sparrow (*Passer domesticus*) nestlings had been shown to be involved in Hale County, Texas.

In 1973 no WE virus isolation was obtained from 15,045 mosquitoes, 428 pools, collected and tested during the period July 11 through September 12, 1973, whereas, there had been 110 isolations of WE virus from mosquitoes obtained in 1972. Strains of St. Louis encephalitis (SLE), Hart Park-Flanders (HP-FLA), and Turlock (TUR) viruses were isolated from mosquitoes both years; the single isolate of a Bunyamwera (BUN) group arbovirus was obtained only in 1973. The number of mosquitoes and the virus isolations for 1972 and 1973 are listed in Tables 1 and 2, respectively. An unidentified viral agent also was obtained from 1 of 21 pools of hemiptera (*Oeciacus vicarius*) that were collected from a nest containing immature house sparrows.

Although no WE isolates were obtained from mosquitoes collected in the Morgan County study area during 1973, there was evidence of WE virus activity among avian hosts at several study sites within the area. Twenty-five of 27 viral agents isolated from nestling birds have been identified as "WE-like" in preliminary laboratory tests; 2 of the isolates from nestling birds are Turlock virus. All of the virus strains obtained from nestling birds were from house sparrows, and the infections were detected during the months of July and August. Table 3 lists the nestling bird study data for the nestling bird collections by species and dates. The serologic conversion ratios determined by the percentage of chickens with WE and SLE hemagglutination-inhibition (HI) antibody at three of the study sites in Morgan County are listed in Table 4.

(R. O. Hayes, D. B. Francy, L. J. Ogden, F. C. Harmston, J. Lazuick, and A. D. Hess)

B. Cameron County, Texas.

No WE virus was isolated from mosquitoes in Cameron County, Texas (Brownsville) during VE epidemic studies in June, July, and August 1971. Coincidental with the arrival of south-bound migratory birds in the study area in September of that year, 34 WE virus isolations were made from _Aedes sollicitans_, Table 5. In October 1971, two WE isolations were made from Texas tortoises, and seven WE isolations were made from five species of mosquitoes. Eight additional WE viruses were isolated from four mosquito species in the Presidio, Texas area in October of the same year, Table 6.

_Culex tarsalis_ is present in the south Texas area but is more abundant in cooler months of the year. No WE virus was isolated from the relatively few specimens of this species collected and tested during that time.

This data suggests that basic WE transmission cycles are somewhat different from those occurring farther north and west, where _C. tarsalis_ is thought to be principally involved, and in the east where _Culiseta melanura_ is primarily involved in enzootic transmission cycles.

The appearance of WE late in the season of 1971 suggested the possibility of continuous transmission of the virus in the Brownsville area during the winter. This area is the only area in the continental United States within the sub-tropical zone, other than south Florida.

Studies were initiated in Cameron County in November 1973 to determine if WE transmission may occur through the winter. A summary of the mosquitoes collected and tested for virus thus far is presented in Table 7, and results of the virus isolation tests are presented in Table 8.

WE virus was isolated from two mosquito species, _Aedes sollicitans_ and _Psorophora confinnis_, in November 1973, but no additional WE isolations have been made from mosquitoes collected in December 1973 and January and February 1974. The _Aedes_ and _Psorophora_ species from which WE had been isolated previously in this area were either present in low numbers or absent during this time period.

Over 800 vertebrate sera collected in December 1973 and January 1974 were tested for WE virus with negative results, Table 9. February 1974 sera are still on test. Numerous vertebrates were recaptured; rates as high as 42 percent were recorded. These sera will be tested for WE Ab and Ab-conversions in the neutralization test as another means of uncovering possible continuous winter transmission of WE.

(W. D. Sudia, R. O. Hayes, V. F. Newhouse, R. McLean, S. Bowen, G. Smith, J. Lazuick, L. J. Ogden, and F. C. Harmston)

## II. Eastern Equine Encephalitis Investigation in New Hampshire and Massachusetts.

### A. New Hampshire.

The first documented epizootic of eastern equine encephalitis (EEE) in New Hampshire occurred during August and September 1973. Large numbers of pheasants at state-owned game farms were striken and numerous horse cases were reported. Equine and pheasant cases occurred in Rockingham County in the southeastern portion of the state adjacent to Massachusetts. The apparent focus of the pheasant epizootic was in the region of the pheasant farm about 5 miles southwest of Exeter, New Hampshire. The equine cases were concentrated in a 100-square mile area around the pheasant farm.

Mosquitoes were collected at selected sites in the outbreak area and tested for arboviruses by personnel of the Vector-Borne Diseases Division. Eastern equine encephalitis virus isolations were made from 5 of the 18 mosquito species collected. Six of the 10 EEE virus strains recovered were from *Culiseta melanura* which is considered the principal enzootic vector of this virus. These recoveries represent the first isolations of EEE virus from naturally infected mosquitoes in New Hampshire. Mosquito species tested and virus strains isolated are summarized in Table 10.

### B. Massachusetts.

In response to a request for laboratory assistance, current testing of mosquitoes and a limited number of vertebrate specimens was provided for the Massachusetts State Health Department Virus Laboratory during an extensive EEE epizootic in pheasants and equines in Massachusetts. Mosquitoes were also collected by members of the Arbovirus Ecology Section sent to Massachusetts to assist with the epizootic.

There were 25,174 mosquitoes submitted in 1,126 pools for virus isolation and identification. From these mosquitoes, 79 strains of WEE virus, 53 strains of EEE virus, and 23 unidentified viruses were recovered. In addition, EEE virus was isolated and identified from 4 of 6 equine brains and 3 of 7 pheasant brains, and WEE virus was recovered from 3 of 10 house sparrow brains submitted for testing. A total of 81 primary virus isolations made in the Mass. State Health Dept. Virus Laboratory were also submitted for reisolation and identification. Reisolation

was successful from 63 of the pools, and 62 of these virus strains were identified as WEE virus. A single strain remains unidentified.

During the outbreak, mosquitoes were sent daily from Massachusetts and generally arrived in Denver the same day they were sent. Specimens were inoculated onto primary duck embryo Vero cell culture monolayers. Isolation and identification of EEE and WEE virus strains was usually complete within 36-48 hours after inoculation. Results were phoned daily to the Mass. State Health Dept. where the information was used to plan insecticidal spray operations on a day-to-day basis.

Mosquito species tested and virus strains isolated are summarized in Table 11.

(D. B. Francy, J. S. Lazuick, R. O. Hayes, and G. C. Smith, J. G. Johnston, Jr., V. F. Newhouse, and W. D. Sudia)

Table 1
Arbovirus isolations from mosquitoes collected during pre-impoundment studies in the Narrows Unit, South Platte River, northeastern Colorado, 1972.

| Mosquito species | No. mosq. tested | No. pools tested | Virus isolations | | | | |
|---|---|---|---|---|---|---|---|
| | | | WE | TUR | SLE | HP-FLA | Unk. |
| *Aedes dorsalis* | 135 | 6 | | | | | |
| *A. hendersoni* | 47 | 13 | 1 | | | | |
| *A. increpitus* | 2 | 2 | | | | | |
| *A. melanimon* | 64 | 4 | | | | | |
| *A. trivittatus* | 842 | 33 | | | | | |
| *A. vexans* | 6,607 | 67 | 2 | | | | |
| *Culex pipiens* | 211 | 5 | | | | | |
| *C. tarsalis* | 12,509 | 142 | 74 | 17 | 3 | 4 | 9 |
| *Culiseta inornata* | 58 | 5 | | | | | |
| *Mansonia perturbans* | 4 | 3 | | | | | |
| Total | 20,479 | 280 | 77 | 17 | 3 | 4 | 9 |

Table 2
Arthropod collections from Morgan County, Colorado and arbovirus isolation test results for the period July 11-September 12, 1973.

| Arthropods | Mosquitoes | (Pools) | Virus isolations* | | | | |
|---|---|---|---|---|---|---|---|
| | | | SLE | BUN | HP-FLA | TUR | Unk. |
| *Aedes dorsalis* | 2,831 | (78) | | 1 | | | |
| *A. hendersoni* | 64 | (10) | | | | | |
| *A. increpitus* | 30 | (3) | | | | | |
| *A. melanimon* | 1,670 | (50) | | | | | |
| *A. sticticus* | 10 | (1) | | | | | |
| *A. trivittatus* | 128 | (13) | | | | | |
| *A. vexans* | 7,110 | (108) | | | | | |
| *Anopheles earlei* | 1 | (1) | | | | | |
| *Culex pipiens* | 1 | (1) | | | | | |
| *C. tarsalis* | 2,980 | (138) | 1 | | 7 | 3 | 1 |
| *Culiseta inornata* | 116 | (22) | | | | | |
| *Mansonia perturbans* | 2 | (2) | | | | | |
| *Psorophora signipennis* | 2 | (1) | | | | | |
| Total | 15,045 | (428) | 1 | 1 | 7 | 3 | 1 |

*Preliminary identification indicates the virus strains isolated are not Group A arboviruses.

Table 3
Arbovirus isolations/nestling bird collections by species and dates during 1973 in Morgan County, Colorado.

| Date | House Sparrow | Barn Swallow | Cliff Swallow | Brewer's Blackbird | Pigeon | Total |
|---|---|---|---|---|---|---|
| June 27 | 0/20 (0%) | | | | | 0/20 |
| July 9-11 | 11/70 (16%) | 0/10 | | 0/12 | 0/5 | 11/97 |
| July 23-25 | 6/78 (8%) | 0/10 | 0/19 | | 0/4 | 6/111 |
| August 7-8 | 9/85 (11%) | 0/16 | | | 0/5 | 9/106 |
| August 20-21 | 1/59 (2%) | 0/4 | | | 0/4 | 1/67 |
| September 10-12 | - | - | - | - | - | - |
| Total | 27*/312 (9%) | 0/40 | 0/19 | 0/12 | 0/18 | 27/401 |

*Two virus strains have been identified as Turlock virus; all other strains are "WE-like" Group A arboviruses.

Table 4
Seasonal transmission indices among chicken flocks at three study sites in Morgan County, Colorado during 1972 and 1973.

| | Percentage of birds with HI antibody | | | |
|---|---|---|---|---|
| | 1972 | | 1973 | |
| Study site* | WE | SLE | WE | SLE |
| Poe | 71 | 18 | 54 | 0 |
| Nichol | 70 | 7 | 58 | 0 |
| Peterson | 43 | 20 | 14 | 14 |

*Flock size ranged between 28 and 31 chickens.

Table 5.
WEE viruses isolated from mosquitoes in 1971 during VEE studies in Brownsville, Texas.

| Species | September | October | Total |
|---|---|---|---|
| Aedes sollicitans | 34 | 3 | 37 |
| taeniorhynchus | | 1 | 1 |
| thelcter | | 1 | 1 |
| Psorophora confinnis | | 1 | 1 |
| discolor | | 1 | 1 |
| Total | 34 | 7 | 41 |

Table 6.
WEE virus isolations from mosquitoes collected in the Presidio area, Texas, October 1971 and April to November 1972.

| | October 1971 | | April-November 1972 | |
|---|---|---|---|---|
| Species | No. mosq. collected | No. WEE isolations | No. mosq. collected | No. WEE isolations |
| A. nigromaculis | 126 | 1 | 103 | 0 |
| vexans | 4,246 | 4 | 6,139 | 2 |
| C. tarsalis | 115 | 0 | 1,102 | 1 |
| P. confinnis | 43 | 1 | 900 | 0 |
| signipennis | 256 | 2 | 233 | 0 |
| 18 other species | 130 | 0 | 4,108 | 0 |
| Total | 4,916 | 8 | 12,585 | 3 |

February 1974 for virus isolation studies.

| Species | 1973 | | 1974 | | |
|---|---|---|---|---|---|
| | November | December | January | February | Total |
| Aedes bimaculatus | 1 (1)* | | | | 1 (1) |
| scapularis | | | 792 (17) | 78 (2) | 870 (19) |
| sollicitans | 608 (20) | 697 (34) | 84 (22) | 45 (10) | 1,434 (86) |
| taeniorhynchus | 160 (7) | 3 (2) | | | 163 (9) |
| thelcter | 27 (6) | 1 (1) | | | 28 (7) |
| vexans | 8 (4) | 6 (2) | 775 (16) | 94 (2) | 883 (24) |
| Anopheles crucians | 16,475 (168) | 16,153 (166) | 10,540 (214) | 197 (5) | 43,365 (553) |
| pseudopunctipennis | 1,702 (21) | 2,506 (30) | 125 (10) | 10 (3) | 4,343 (64) |
| quadrimaculatus | 840 (13) | 685 (13) | 480 (16) | 9 (4) | 2,014 (46) |
| Culex coronator | 18 (4) | 89 (6) | 1,143 (23) | 75 (2) | 1,325 (35) |
| (Mel.) erraticus | | 8 (4) | 188 (13) | 10 (3) | 206 (20) |
| (Mel.) sp. | 1,073 (15) | 47 (5) | | 11 (1) | 1,131 (21) |
| nigripalpus | | 27 (4) | 10 (2) | | 37 (6) |
| quinquefasciatus | | 12 (1) | 31 (2) | | 43 (3) |
| restuans | 1 (1) | 66 (6) | | | 67 (7) |
| salinarius | 3,042 (36) | 3,764 (42) | 9,629 (197) | 684 (17) | 17,119 (292) |
| tarsalis | 15 (6) | 143 (12) | 80 (14) | 19 (3) | 257 (35) |
| Culiseta inornata | 2 (2) | 304 (8) | 15 (5) | | 321 (15) |
| Deinocerites mathesoni | 17 (5) | 7 (1) | 8 (3) | 3 (2) | 35 (11) |

Table 7.
Summary of mosquitoes collected in south Texas from November 1973 through February 1974 for virus isolation studies.
(continued)

| Species | 1973 | | 1974 | | Total |
|---|---|---|---|---|---|
| | November | December | January | February | |
| Mansonia titillans | 253 (8)* | 56 (7) | 11 (6) | | 320 (21) |
| Psorophora ciliata | 1 (1) | | | | 1 (1) |
| confinnis | 119 (9) | 12 (5) | 1 (1) | 1 (1) | 133 (16) |
| ferox | 2 (1) | 1 (1) | | | 3 (2) |
| Uranotaenia lowii | 34 (5) | 4 (1) | | | 38 (6) |
| sapphirina | 2 (1) | | | | 2 (1) |
| Total | 24,400 (334) | 24,591 (351) | 23,912 (561) | 1,236 (55) | 74,139 (1,301) |
| Trap nights | 62 | 160 | 176 | 138 | 536 |
| Average/trap night | 394 | 154 | 136 | 9 | 138 |

*Number mosquitoes collected (number pools tested).

Table 8.
Virus isolations from mosquitoes in south Texas during winter studies, 1973-74.

| Species | November 1973 | January 1974 |
|---|---|---|
| | WE | Unidentified virus |
| *Aedes sollicitans* | 5 | |
| *Psorophora confinnis* | 1 | |
| *Anopheles crucians* | | 1 |
| *Culex salinarius* | | 6 |
| Total | 6 | 7 |

Table 9.
Summary of vertebrates collected in south Texas from December 1973 through February 1974 for virus isolations and antibody studies.

| | Number collected | Number recaptured | % recaptured |
|---|---|---|---|
| December 1973 | | | |
| Wild mammals | 223* | 5 | 2 |
| Wild birds | 127 | 12 | 9 |
| Domestic animals | 96 | -- | |
| Texas tortoises | 24 | 10 | 42 |
| Other reptiles | 5 | -- | |
| Total | 475 | | |
| January 1974 | | | |
| Wild mammals | 169 | 44 | 26 |
| Wild birds | 157 | 19 | 12 |
| Texas tortoises | 2 | -- | |
| Total | 328 | | |
| February 1974 | | | |
| Wild mammals | 200 | 83 | 42 |
| Wild birds | 85 | 19 | 22 |
| Texas tortoises | -- | -- | |
| Other reptiles | 1 | -- | |
| Total | 286 | | |

*Two virus strains, positive in Vero cells, positive in SM, and negative in DEC, as yet unidentified, were isolated from *N. micropus* in December 1973.

Table 10. Rockingham County, New Hampshire Arbovirus Isolations from Mosquitoes Collected August 23-24, 1973.

| Species | Number | Pools | Virus Isolations EEE |
|---|---|---|---|
| *Aedes canadensis* | 66 | 4 | |
| *A. cantator* | 30 | 4 | |
| *A. cinereus* | 27 | 5 | |
| *A. excruians* | 8 | 2 | |
| *A. fitchii* | 3 | 2 | |
| *A. sollicitans* | 5 | 3 | |
| *A. triseriatus* | 24 | 5 | |
| *A. vexans* | 458 | 11 | 1 |
| *Anopheles punctipennis* | 59 | 7 | 1 |
| *An. quadrimaculatus* | 36 | 5 | 1 |
| *Coquilletidia perturbans* | 45 | 6 | |
| *Culex restuans* | 14 | 4 | |
| *C. salinarius* | 143 | 7 | 1 |
| *Culiseta melanura* | 323 | 10 | 6 |
| *C. morsitans* | 49 | 8 | |
| *Orthopodomyia signifera* | 1 | 1 | |
| *Uranotaenia sapphorina* | 55 | 5 | |
| Totals | 1,346 | 89 | 10 |

Table 11.
Mosquitoes tested and virus strains recovered during eastern equine encephalitis epizootic in Massachusetts, 1973.

| Mosquito species | No. mosq. tested | No. pools tested | Virus isolations WE | EE | Unident. |
|---|---|---|---|---|---|
| Aedes canadensis | 679 | 59 | 1 | | |
| A. cantator | 107 | 20 | | | |
| A. cinereus | 265 | 28 | | 1 | |
| A. fitchii | 1 | 1 | | | |
| A. sollicitans | 32 | 10 | | | |
| A. triseriatus | 127 | 29 | | | |
| A. trivittatus | 1 | 1 | | | |
| A. vexans | 1,552 | 85 | | 1 | |
| Anopheles spp. | 1 | 1 | | | |
| An. punctipennis | 227 | 26 | | | |
| An. quadrimaculatus | 244 | 38 | | | |
| An. walkeri | 20 | 6 | | | |
| Culex spp. | 49 | 14 | | | 1 |
| C. pipiens | 212 | 45 | 1 | | |
| C. restuans | 201 | 56 | 1 | | |
| C. salinarius | 3,073 | 121 | | | 1 |
| C. territans | 16 | 13 | | | |
| Culiseta melanura | 16,690 | 420 | 75 | 47 | 21 |
| C. minnesotae | 8 | 4 | | | |
| C. morsitans | 133 | 31 | | 2 | |
| Psorophora ferox | 1 | 1 | | | |
| P. cyanescens | 1 | 1 | | | |
| Uranotaenia sapphirina | 1,049 | 66 | 1 | 1 | |
| Coquillettidia perturbans | 383 | 47 | | 1 | |
| **Total** | 25,174 | 1,126 | 79 | 53 | 23 |

REPORT FROM THE ENVIRONMENTAL AND ECOLOGY RESEARCH GROUP
DUGWAY PROVING GROUND, UTAH

## Arbovirus Surveillance of West Central and Southern Utah

### Bonneville Basin

A total of 25,001 arthropods (mosquitoes and *Culicoides*) in 292 pools were collected and assayed for arboviruses. Forty-three isolations were made. To date twenty-one have been identified as California Group viruses by the suckling mouse neutralization test. All isolates were from *Aedes* pools. Non sensitivity of one additional isolate to sodium deoxycholate has eliminated it from arbovirus classification.

### Southern Utah

A total of 5,341 arthropods, including mosquitoes and *Culicoides* gnats, were assayed in suckling mice. Five isolations have been made, one of which was identified as the western encephalitis virus; this was from a pool of *Culex tarsalis* mosquitoes from Bloomington, near St. George, Utah. Four isolates remain for identification.

(George T. Crane, Robert E. Elbel and Keith L. Smart)

| Species | Blue Lake a/p (i) |
|---|---|
| <u>Aedes</u> <u>dorsalis</u> | 5186/53 (15) |
| <u>Aedes</u> <u>melanimon</u> | |
| <u>Aedes</u> <u>nigromaculis</u> | |
| <u>Aedes</u> <u>vexans</u> | |
| <u>Culiseta</u> <u>inornata</u> | 5/ 2 |
| <u>Culex</u> <u>tarsalis</u> | 1266/14 |
| <u>Culex</u> <u>erythrothorax</u> | 19/ 2 |
| <u>Culicoides</u> <u>variipennis</u> | |
| TOTALS | 6476/71 (15) |

a = number of arthropods assayed
p = number of pools assayed
i = number of isolations

| Callao a/p (i) | Fish Springs a/p (i) | Totals a/p (i) |
| --- | --- | --- |
| 2285/27 (4) | 8989/91 (22) | 16460/171 (41) |
| 204/ 7 | | 204/ 7 |
| 49/ 2 | | 49/ 2 |
| 94/ 5 | | 94/ 5 |
| 197/ 8 | 21/ 3 | 224/ 13 |
| 3425/38 | 441/ 6 | 5132/ 58 |
| 6/ 1 | 1547/17 | 1572/ 20 |
| 155/ 3 (1) | 1112/13 ( 1) | 1267/ 16 ( 2) |
| 6415/91 (5) | 12110/130(23) | 25001/292 (43) |

| Species | Beaver Wash | Golf Course | Middleton | Bloomington | Totals |
|---|---|---|---|---|---|
| | a/p (i) | a/p (i) | a/p (i) | a/p (i) | a/p (i) |
| *Aedes dorsalis* | 12/ 3 | 3/1 | 7/ 2 | 336/ 4 | 358/10 |
| *Aedes vexans* | | 8/1 | | 3398/32 | 3406/33 |
| *Anopheles franciscanus* | 213/ 4 | | 297/ 5 | 11/ 1 | 521/10 |
| *Anopheles freeborni* | 5/ 1 | | 11/ 3 | 26/ 3 | 42/ 7 |
| *Culiseta inornata* | 54/ 2 (1) | 8/2 | 4/ 3 | 50/ 2 | 116/ 9 (1) |
| *Culiseta incidens* | | 2/1 | | | 2/ 1 |
| *Culex tarsalis* | 60/ 3 (1) | 113/2 | 33/ 2 | 147/ 3 (2) | 353/10 (3) |
| *Culex erythrothorax* | 2/ 1 | 30/2 | 6/ 1 | 17/2 | 55/ 6 |
| *Culicoides variipennis* | | | 278/ 3 (1) | 210/4 | 488/ 7 (1) |
| TOTALS | 346/14 (2) | 164/9 | 636/19 (1) | 4195/51 (2) | 5341/93 (5) |

a = number of arthropods assayed
p = number of pools assayed
i = number of isolations

Surveillance for mosquito-borne encephalitis during the 1973 season has again confirmed the persistence of western encephalitis (WEE) and St. Louis encephalitis (SLE) viruses in their natural vectors and hosts in many areas of the State. However, vector control and equine immunization programs kept human and equine cases of disease at the low levels characteristic of the past two decades.

Fifty-six clinically suspect equine cases were reported to the Department, but only 2 cases of WEE were actually documented by serologic tests: a 1-year-old unvaccinated horse from Yolo County, onset August 12; and a 4-month-old colt from Shasta County, onset September 4.

No human cases of WEE were detected during the year; and there was no evidence of Venezuelan equine encephalitis in California, nor any resurgence of this disease in the Central and Northern areas of Mexico which were affected in previous years. However, 5 cases of SLE were confirmed: (1) a 62-year-old woman from Riverside County, onset July 19; (2) a 17-year-old girl from Kern County, onset August 12; (3) a 29-year-old woman from San Joaquin County, with onset August 24, who visited in Butte County shortly before illness, but whose mosquito exposure was most likely in her home environment; and 2 cases in San Diego County detected by the local Public Health Laboratory and confirmed by the State Virus Laboratory--(4) a 12-year-old boy with onset September 4 and exposure most likely in a San Diego suburban area; and (5) a 31-year-old man presumably exposed in Imperial County, onset October 10. Two additional suspect cases, a 22-year-old man from Butte County and a 23-year-old man from Riverside County, showed low complement-fixing SLE antibody titers and further laboratory tests are being done. In SLE particularly, the CF antibody may be slow to develop, and neutralization, hemagglutination-inhibition, and indirect fluorescent antibody titers may be necessary to establish the diagnosis. No fatality from WEE or SLE has been recorded in the State since 1962.

The mosquito testing program is carried on by the State Vector Control Section, Local Mosquito Abatement Districts, and the State Virus Laboratory. Of 4,842 samples collected in 1973 by this program, the yield was 275 virus isolates, the highest recovery rate in recent years. There were 97 isolates of WEE virus, and 75 of SLE virus, mostly from Imperial County where specimen collection was concentrated because of concern that VEE might recrudesce and move northward from Mexico. In addition, 75 strains of Turlock virus, 26 of a "new" virus closely related or identical with Hart Park virus, and 3 strains of California encephalitis virus, 3 of Main Drain virus, 2 Bunyamwera group viruses, and 1 as yet unidentified virus were isolated.

Despite the frequency of Turlock and Hart Park virus isolations from *Culex tarsalis* (the common and efficient vector for WEE and SLE viruses), little or no evidence of human or equine illness or even of infection has been obtained in California. And despite the reappearance of California encephalitis strains of virus in mosquitoes, after many years absence, no human cases of disease have been detected.

A total of 25 cases of Colorado tick fever were confirmed in 1973, the largest number of cases since recordkeeping began in 1954. From 1954 to 1973, 165 laboratory-proven cases have been recorded.

(R.W. Emmons)

REPORT FROM THE DIVISION OF MEDICAL MICROBIOLOGY
UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, CANADA

Culiseta inornata mosquitoes, which were wild-caught in the Yukon Territory during May 1973, contained 4.5 $log_{10}$ mouse $LD_{50}$ of infectivity in salivary glands and thoraces 194 days after intrathoracic injection with 30 mouse $LD_{50}$ of a Yukon 1971 isolate of California encephalitis (CE) virus snowshoe hare subtype, when mosquitoes were held at 40°F during the initial 3 months, and 30°F thereafter.

Laboratory bred Aedes aegypti mosquitoes transmitted virus by biting suckling mice 34 days after injection with 1 mouse $LD_{50}$ of a Yukon 1972 mosquito isolate of CE virus, 72-Y-144, following incubation at 55°F. The minimum infectivity dose of this CE strain by intrathoracic injection was 0.01 mouse $LD_{50}$. Transmission by biting mice was also demonstrated by A. aegypti after incubation for 28 days at 75°F and 34 days at 55°F, when mosquitoes were injected with 100 mouse $LD_{50}$ of a 1973 Yukon CE isolate 73-Y-347. These mosquitoes transmitted virus after 28 days incubation at 75°F following imbibing of 30 mouse $LD_{50}$ of the 73-Y-347 strain from blood of suckling mice rendered viremic immediately beforehand by intracerebral injection.

Transmission of a Southwest Pacific isolate of dengue-2 virus, NC-6, to suckling mice by bites of A. aegypti was effected 32 days after mosquitoes imbibed 1 to 10 mouse $LD_{50}$ of virus in blood of suckling mice rendered viremic immediately beforehand by intracerebral injection of first mosquito passage material, both when mosquitoes were incubated at 70°F, and also at 55°F. Infectivity titers of the NC-6 strain of dengue-2 virus attained 4.0 log mouse $LD_{50}$ in salivary glands and thoraces of mosquitoes after 41 days incubation at 55°F, following imbibing of 1 to 10 mouse $LD_{50}$ of zero passage material from a blood-soaked cotton pledget.

(D.M. McLean)

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

Previous studies in our laboratory have shown that dengue virus replicates in lymphoid tissue in experimentally infected rhesus monkeys and in cultures of peripheral blood leukocytes from dengue immune monkeys but not those from non-immune animals. The results of subsequent studies on virus leukocyte interactions and dengue pathogenesis in immuno-suppressed monkeys are briefly summarized.

Studies on *in vitro* attributes of dengue virus - primate leukocyte interactions. Peripheral leukocytes from rhesus monkeys immune to dengue types 1, 2, 3 or 4 supported growth of d1 and d4 as well as d2. *In vitro* leukocyte permissiveness to dengue virus infection did not develop until two to three weeks after monkey inoculation with homologous or heterologous virus. Virus permissiveness of leukocytes in d1, d2, d3 or d4 immune monkeys persisted for up to two years or longer, but tended to decrease with time in some monkeys immune to d4.

Lymph node cells, whether from immune or non-immune monkeys failed to support dengue virus growth or did so to a lesser extent than did peripheral leukocytes. Thymus cells and lung macrophages did not support virus growth. Spleen and bone marrow cells from immune monkeys supported virus growth while those from non-immune animals did not.

Stimulation of peripheral leukocytes from non-immune monkeys with non-specific mitogens (phytohemagglutinin, concanavalin A, poke week mitogen, streptolysin O) did not induce the cells to become permissive to virus infection. PHA stimulation of permissive leukocytes from dengue immune monkeys suppressed virus replication.

Attempts to define the specific cell population (or populations) in which dengue virus replicates *in vitro* are still in a preliminary stage. In a series of experiments we have failed to demonstrate dengue virus replication in relatively pure cultures of strongly glass adherent cells (macrophages) from either monkeys or man. Completely non-adherent cells likewise failed to support virus growth, whereas weakly adherent cells regularly supported virus replication to high titer. These results suggest that the permissive cell population may be included in the B-lymphocyte component. Treatment of permissive leukocytes with rabbit anti-monkey immunoglobulin or Trypsin reduced permissiveness to dengue infection *in vitro*. Also, cells in eluates from columns containing anti-monkey immunoglobulin derivatized agarose beads exhibited reduced capacity to support virus growth. These data are consistent with the hypothesis that the permissive cell population contains cells with surface immunoglobulins.

In a more direct approach, spleen cells from an immune monkey were infected with d2 at a MOI of 1.0 and incubated at 37°C for 2 or 6 hours, then stained for presence of surface immunoglobulin with fluorescein-tagged anti-monkey immunoglobulin. Cells treated with virus diluent served as controls. Approximately 50% of the spleen cells exhibited distinct membrane fluorescence before

and after 2 or 6 hours of incubation with d2 or control antigen. Distinct polar fluorescence (cap formation) was observed in 10%-20% of the d2-treated cells after 6 hours incubation. No polar fluorescence was seen at 2 hours or in control cultures. After four days incubation the virus titer in infected culture was $3.5 \times 10^4$ pfu/ml by plaque assay.

Studies currently in progress suggest that the permissive cell population in freshly prepared leukocytes is very small, even in cells from immune animals. Infectious center assays of permissive cells immediately after adsorption of d2 for 90 minutes indicate that less that 10 cells/million were initially infected. The number of infected cells increased to 75/million on day 1, 2000 cells/million on day 3 and 3000/million on day 4 of culture. In non-permissive cells less than 1 cell/million was detected immediately after adsorption.

Dengue virus production *in vivo* in immuno-suppressed monkeys. A non-immune monkey was treated with cyclophosphamide (cytoxan) at doses sufficient to reduce and maintain its white blood cell count at approximately $2000/mm^3$ (normal count approximately $18{,}000/mm^3$). The low WBC count was maintained for one week and the monkey was inoculated subcutaneously with $2 \times 10^5$ pfu of dengue 2. Maintenance doses of cytoxan were continued for 12 days post-infection. Blood was drawn daily for 15 days post-infection for measurement of viremia. The monkey was viremic from day 3 through day 14 with a peak titer on day 6 of $1.85 \times 10^4$ pfu/ml of plasma. The viremia titer was greater than 1000 pfu/ml of plasma for 9 consecutive days (days 4 to 12). There was no detectable antibody response until after the cessation of cytoxan treatment. To our knowledge this is the longest and most severe dengue viremia ever recorded in a non-immune monkey.

In a second experiment, a dengue 4 immune monkey was similarly immunosuppressed with cytoxan and challenged with d2. Cytoxan treatment was continued for 17 days post d2 challenge. This monkey was viremic from day 2 through day 28 (the last day tested) post-infection with highest titers (>1000 pfu/ml) occurring on days 17 through 21. Dengue 2 HAI antibody titer was 1/10 three weeks post infection (the last day tested to date).

These interesting observations suggest that in dengue infected rhesus monkeys the elimination of circulating virus is closely related to the production of type specific dengue antibody. These data, however, do not eliminate the possibility that other immune mechanisms play a role in virus elimination in natural infections. We are currently studying the effects of other immuno-suppressants (azathioprine and anti-thymocyte serum) on experimental dengue infection in non-immune and mono-immune monkeys and attempting to determine where and in what cell populations the virus is replicating.

(Nyven J. Marchette and Scott B. Halstead)

1. Hemagglutination inhibiting and neutralizing antibodies for Japanese encephalitis virus in mothers and cord bloods of their newborns living in both Tokyo and Sapporo (Hokkaido).

In 1937 Kuriyama et al. described that out of 32 cord bloods collected in Tokyo, 25 (80%) were positive by mouse-neutralization test (MN test) against Kalinina strain (one of Japanese encephalitis (JE) virus) while 5 cord bloods collected in Hokkaido were negative. In 1950, out of 10 paired serums from mothers and cord bloods of their newborns living in Tokyo, 8 mothers without history of JE vaccination and 7 cord bloods were found positive by MN test (Kitaoka and Miura). In consideration of no large outbreak of JE epidemic occurring in the past several years no markedly subclinical infection would have been expected to occur in inhabitants at the JE endemic area such as in Tokyo. Accordingly the immune state of the young mothers acquired by natural subclinical infection are presumably not so high as before.

The paper presents how a low level of the immune state of young mothers and their newborns in Tokyo compares with that in the Sapporo subendemic area (Kitaoka and Miura). As given in Table 1, 51 paired blood samples from mothers and cord bloods of their newborns were collected through the courtesy of Dr. Kobayashi, Director, the Nihon Red Cross Maternal Hospital, in 1970, and 13 paired samples were collected in Sapporo by the authors in 1970. These mothers in both Tokyo and Sapporo were selected from among mothers without history of JE vaccination, especially mothers in Sapporo who had not traveled in the JE endemic area in Honshu during the summer season. The hemagglutination inhibition (HI) test was carried out according to a modified microtiter method of Clarke and Casals ; and both antigens (Nakayama-NIH and JaGAr-01) were used because of a little variety of antigenicity in the HI test between them as reported by Okuno et al. and Kitaoka. The neutralization test (Nt) was carried out by a 50% plaque reduction assay method in chick embryo cell monolayer culture. Out of 51 paired samples in Tokyo, 23 each of mothers and newborns were HI-positive (45%) for Nakayama-NIH antigen and 19 each HI-positive (37%) for JaGAr-01 antigen. The antibodies were found to be 2-mercaptoethanol (2-ME) resistant. Contrary to those in Tokyo, 2 of 13 mothers in Sapporo were HI-positive (15%) for both Nakayama-NIH and JaGAr-01 antigens, and one cord blood was HI-positive (8%) for both antigens. These antibodies were also 2-ME resistant.

As for Nt antibody, 34 of 47 mothers (72.3%) and 28 of 47 cord bloods (49.6%) were positive for Nakayama-NIH antigen and 27 of 47 mothers (57.5%) and 24 of 47 cord bloods (51%) were positive for JaGAr-01 antigen in Tokyo while in Sapporo 9 of 13 mothers (69.2%) and 8 of 13 cord bloods (61.5%) were positive in both HI and Nt antibodies for both Nakayama-NIH and JaGAr-01 antigens, respectively. In consideration of the Sapporo area being a subendemic area, the fact that the mothers living in Sapporo (and their newborn cord bloods) gave lower HI-positive rates than those in the Tokyo

endemic area suggests that the Sapporo area is still a lower endemic area than Tokyo. It was unexpected, however, that Nt-positive rates of mothers and their newborn cord bloods were not so significantly different between Tokyo and Sapporo. It might be due to difference in persistence of detectable and accumulated Nt antibodies resulting from subclinical infection in mothers living in Tokyo and Sapporo.

After all, it is pointed out that the Nt-antibody rates of mothers, especially their newborns, in Tokyo in May 1970 were significantly lower than those in 1950 and 1957, and also lower compared with the Nt-antibody positive rate (60.9%) of age group (0-5) among 2,136 inhabitants in Miyagi, Gumma, Hyogo, Shimane, Fukuoka, Nagasaki Prefectures and Iki island, 1967.

From the foregoing it can be said that the maternal antibody rate in Sapporo was surely lower than that in Tokyo. Furthermore, the maternal Nt antibody rate in Tokyo has been decreasing year after year since 1937. On the contrary, the Sapporo has apparently become more endemic during the period 1937-1970.

In conclusion, it is recommended that mothers living in Tokyo should be immunized or revaccinated to boost the antibody level not only for their own benefit but that of their newborns as well, to protect against JE virus infection.

(M. Kitaoka, T. Ogata, and A. Shimizu, National Institute of Health, Tokyo, Japan; I. Tomizawa and T. Akiba, the Sapporo Municipal Hospital, Sapporo, Japan)

Table 1.

HI and NT antibodies in mothers and cord bloods of their new borns in Tokyo and Sapporo

| Area | | Samples | Date of collection | Antigen used Nakayama-NIH | JaGAr-01 |
|---|---|---|---|---|---|
| Tokyo | HI | mothers | May, 1970 | 23/51=45% | 19/51=37% |
| | | cord bloods | | 25/51=48% | 19/51=37% |
| | NT | mothers | May, 1970 | 34/47=72.34% | 27/47=57.45% |
| | | cord bloods | | 28/47=49.57% | 24/47=51.11% |
| Sapporo | HI | mothers | March, 1970 | 2/13=15% | 2/13=15% |
| | | cord bloods | | 1/13=8% | 1/13=8% |
| | NT | mothers | March, 1970 | 9/13=69.2% | 9/13=69.2% |
| | | cord blood | | 8/13=61.5% | 8/13=61.5% |

2. Serological survey of arboviruses discovered in Japan in field rodents.

In order to elucidate the ecology of Japanese encephalitis (JE) virus, the susceptibility of JE virus for the field rodents had been reported, that is, Microtus montebelli suffered from JE infection and died following intracerebral inoculation of JE virus and the virus could pass from brain to brain of rodent, while Apodemus speciosus speciosus could survive without any symptoms following the intracerebral inoculation (Mitamura and Kitaoka).

Apoi virus, one of the group B arboviruses, was isolated from rodents trapped in Hokkaido (Saeki et al. and Kitaoka et al.). On the other hand, JE virus (group B; Mitamura et al., Kitaoka et al. and Oya et al.), Getah (group A; Oya et al.), Akabane and Aino (Simbu group; Oya et al. and Takahashi et al.) and Batai (Bunyamwera group; Kobayashi et al.) viruses were isolated from mosquitoes in nature in Japan. In order to determine the role of the field rodent in the ecology of these arboviruses in Japan, the serological survey was carried out on the field rodents trapped at scattered selected areas in Japan. The results obtained so far are briefly summarized as follows: 4 of 23 Apodemus speciosus speciosus trapped at Kunisaki Peninsula, Kushu and 2 of 24 Microtus montebelli trapped at Gosen, Niigata Prefecture were HI-positive for JE antigen, while 3 of 52 Clethrionomys rubocasus bedfordiae were HI-positive for both JE and Apoi antigens and another 3 of 52 were found HI-positive for Apoi antigen only. The survey on the other viruses is still in progress. It can be said that the field rodent does not appear to play an important role in the epidemiology of JE virus.

(M. Kitaoka, T. Ogata, and A. Shimizu)

3. Thinlayer chromatography of brain lipids infected with two of Japanese encephalitis, chikungunya and dengue type 1 viruses.

In comparing two distinct strains of Japanese encephalitis (JE) viruses, the Nakayama and Jath-160, there were significant differences noted in the structure of the virions, the lipid and protein constituents, their biological properties of hemagglutinating activity and their neurovirulence to adult mice by intraperitoneal (ip) route[1].

Thinlayer chromatography of brain lipids of suckling mice infected with JE, chikungunya and dengue type 1 viruses commonly revealed no marked spots of phrenosine (Rf = 3.0) and sphingomyelin (Rf = 0.8) as compared with lipid components of normal mouse brains. Concerning kerasine (Rf = 3.5), it was not detectable in the brain extracts infected with JE virus Jath-160 strain (which had high neurovirulence by ip route) but showed a weak spot in JE virus Nakayama strain (with low neurovirulence by ip route), chikungunya and dengue type 1 viruses.

These results suggest that the differences in brain lipids following arbovirus infections may explain the neurotropism of virus infection by peripheral inoculation.

(M. Kitaoka, C. Nishimura*, and A. Shimizu)

*Kitasato University, School of Pharmaceutical Sciences, Tokyo)

REFERENCE:

Nishimura, C., Nomura, M., and Kitaoka, M. (1968): Comparative studies on the structure and properties of two selected strains of Japanese encephalitis virus, Jap. J. Med. Sci. Biol., 21, 1-10.

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

1. Effect of amino acids on growth of Singh's Aedes albopictus cells and Japanese encephalitis virus.

Attempts were performed to adapt Singh's A. albopictus (SA) cell line to basal growth medium of Eagle's minimal essential medium (MEM) with 10% calf serum. As a result of this adaptation, we found that serine (and proline) were required for the growth of SA cells. On the other hand, maximal growth of JEV depended on the presence of proline (and glycine) in the medium for virus growth (Table 1.). However, growth of JEV in BHK21 cells did not depend on these amnio acids.

2. Proline deficiency and growth inhibition of Japanese encephalitis virus in Singh's A. albopictus cells.

By proline deficiency viral antigen formation as well as virus-specific RNA synthesis was inhibited in Singh's A. albopictus (SA) cells infected with JEV. On the other hand, adsorption and penetration of infecting JEV were not affected by omitting proline from medium for virus adsorption or by predepletion of proline from the cells before virus inoculation. Growth of JEV was inhibited by proline deficiency even when infective JEV-RNA was inoculated to the cells. Meanwhile, synthesis of Sindbis virus-specific RNA and SA cell RNA were not affected so much by proline deficiency. These results seemed to support the idea that there are some proline-dependent steps in the replication of JEV in SA cells and these steps exist after exposure of infecting virion RNA and before progeny viral RNA synthesis.

3. Effect of L-azetidine-2-carboxylic acid on the growth of Japanese encephalitis virus.

The experiment was performed to see whether there are any proline-dependent steps for the replication of JEV also in BHK21 cells. By adding azetidine-2-carboxylic acid, a proline analogue, at 100 μg/ml before and after virus inoculation, 20 hrs' yield of JEV in BHK21 cells was reduced. However, those of Sindbis and Chikungunya viruses were not affected. Inhibitory effect of L-azetidine-2-carboxylic acid was reversed by equimolar amount of L-proline.

4. Fluorescent antibody staining of Singh's A. albopictus cells infected with JEV.

JaOH-0566 strain of JEV with a passage history in MK and BHK21 cells grows to high titer in Singh's A. albopictus (SA) cells. Viral antigen formation in infected cells was followed by direct fluorescent antibody technique. On the first day after infection, viral antigen begins to appear at a certain perinuclear region, and then spread to entire perinuclear region diffusing into cytoplasm. On the second day almost all the cells were

positive with fluorescence around the nucleus diffusing into cytoplasm. The high percentage of fluorescent positive cells seems to correspond with the high virus yield in this system. By omitting proline from the medium for virus growth, both the number of fluorescent positive cells and the amount of viral antigen in a single cell were reduced. The latter seemed to be confined to a certain perinuclear region. This result seemed to correspond with the reduced virus yield in this system by proline deficiency.

(A. Igarashi, F. Sasao, and K. Fukai)

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Igarashi, A., F. Sasao, and K. Fukai, 1973. _Biken J._ 16: 95.

Igarashi, A., F. Sasao, and K. Fukai, 1974. _Biken J._ 17: in press.

Table 1. Effects of omitting each of the 7 "non-essential" amino acids on the growths of Singh's *A. albopictus* cells and JEV

| Amino acid omitted | Nuclear count of the cells* ( $10^5$/0.5 ml ) | Maximal titer of JEV** ( $10^8$ PFU/ml ) |
|---|---|---|
| None | 3.8 ± 0.2 | 4.69 ± 0.56 |
| All 7 | 0.9 ± 0.3 | 0.53 ± 0.16 |
| Alanine | 3.9 ± 0.4 | 4.74 ± 0.95 |
| Asparagine | 4.2 ± 0.7 | 4.53 ± 1.04 |
| Aspartic acid | 4.2 ± 1.0 | 6.45 ± 0.75 |
| Glutamic acid | 3.9 ± 0.5 | 4.45 ± 0.52 |
| Glycine | 3.9 ± 1.1 | 3.25 ± 0.50 |
| Proline | 3.5 ± 0.4 | 0.54 ± 0.10 |
| Serine | 1.1 ± 0.3 | 4.25 ± 0.70 |

Average of triplicate experiments with SE.

* Sampled 7 days after cell transfer in basal growth medium ( 10 % calf serum in MEM ) supplemented with 7 "non-essential" amino acids ( 0.1 mM each ).

** Sampled until 7 days after virus inoculation. Incubated at 28 C with 2 % calf serum in MEM supplemented with 7 "non-essential" amino acids ( 0.1 mM each ).

## Growth of certain arboviruses in human embryo organ cultures

Human embryo tissues obtained by the artificial abortion of medical indications (without apparent signs of infectious diseases) were cultivated by the Rose chamber culture method at 37 C in a $CO_2$ incubator. Culture medium consisted of 199 solution plus fetal calf serum (20%) without antibiotics. Chikungunya (African strain) and dengue type 1 (Mochizuki strain) viruses were examined for capacity of multiplying in the cultures of kidney, liver and chorion. Preliminary results so far obtained indicated that significant rises of infectivity titers of CHIK virus (assayed by the plaque method in BHK-21 cells under methyl cellulose overlay media) were noted in the liver cultures. The same titers of growth in the kidney or chorion cultures were comparatively low, under the conditions studied. Further experiments to elucidate the growth patterns of certain human-pathogenic arboviruses, particularly those believed to be involved in hemorrhagic fever, are being planned.

This study is carried out in collaboration with Drs. H. Nishimura and Y. Yasuda, of the Anatomy Department, Kyoto University School of Medicine.

(T. Matsumura and S. Hotta)

## REPORT FROM THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH, BRISBANE, AUSTRALIA

This preliminary note refers to an epidemic of Murray Valley encephalitis in south-eastern Australia. An epidemic involving about 40 cases in the Murray-Darling basin in 1951 is the basis of most of our present knowledge of Murray Valley encephalitis. Small epidemics in that area occurred in 1956 and 1971, but otherwise the virus has been shown active only in the tropical north of Australia and New Guinea. It was therefore of both public health and research interest when cases of encephalitis occurred in January 1974 at several centres along the Murray River. So far 12 patients have given serological evidence of infection with MVE virus and others are still under investigation. Three patients died. Several Australian laboratories are carrying out field studies--a team led by Dr. Ian Marshall from the Australian National University, Canberra, made extensive collections of mosquitoes and birds in southern New South Wales and northern Victoria, and additional mosquitoes were collected by Professor David Lee of the School of Public Health and Tropical Medicine, University of Sydney. Studies by this Institute at Charleville in south-west Queensland, show extensive seroconversion in domestic fowls; group B virus strains not yet fully identified were isolated from mosquitoes (*Culex annulirostris*) and sentinel chickens.

Previous epidemics were shown (by Anderson and Miles) to follow certain patterns of spring rainfall in northern Australia. On this occasion there was heavy rain in the Murray Valley itself in the latter half of 1973, with heavy rain further north at the end of that year and early 1974. A closer analysis of rainfall patterns is planned.

(R.L. Doherty, J.G. Carley, B.H. Kay, C. Filippich)

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

Ross River virus investigations.

The major epidemic zone of "epidemic polyarthritis with rash" (Ross River virus) is along the major river systems of the inland plains of New South Wales. The presumed causative agent has not been recovered from patients, mosquitoes or wildlife during epidemics in this zone; Doherty's group working in north Queensland has recovered Ross River virus (RRV) from mosquitoes collected on both coasts of Cape York Penninsula, from the blood of an aboriginal girl who did not develop polyarthritis, and, unexpectedly, from the bloods of 3 birds. We have recovered strains of RRV from mosquitoes collected on the coast of New South Wales and have demonstrated minor antigenic differences between these and those recovered in north Queensland, which, with other evidence, prompted us to postulate that the virus was enzootic in parts of temperate coastal New South Wales.

In April 1973, (the antipodean autumn), a collecting trip was made through western N.S.W. as an initial step in a proposed ongoing project to find (a) whether RRV can be recovered in epidemic areas in non-epidemic years, (b) to recover RRV from mosquitoes when a major epidemic occurs and (c) to compare the biologic and antigenic properties of RRV strains obtained with the types already defined from queensland and coastal N.S.W.

Population centres were generally avoided, collecting sites being chosen whenever possible on remote river banks, billabongs and swamps associated with the Macquarie, Paroo, Darling, Murray and Murrumbidgee rivers. The catches from each site were highly variable. Processing of the material has not yet been completed but 16 viruses have so far been isolated (Table 1).

Brigalow Station is on the Macquarie River at approximately 29°55'S., 146°00'E. and close to an extensive system of marshes. In the table the sites are listed from the north of the state at the top to the south at the bottom. The most southerly isolation to date is from near Menindee at 32°20'S.

Analysis of the RRV strains is not yet complete. It is of interest that the Mapputta group strains were recovered from *Anopheles annulipes*; the catalogued prototype Mapputta virus was from *Anopheles meraukensis* and Trubanaman from *Anopheles annulipes*, both from north Queensland. None of the uncharacterised viruses appears to be MVE virus.

1974 epidemic of Murray Valley encephalitis.

The pursuit of the RRV project has been interrupted by the outbreak of Murray Valley encephalitis to be reported by Dr. Ralph Doherty. A hastily mustered field team comprising ornithologists from the Division of Wildlife Research, C.S.I.R.O., and former and present virologists and epidemiologists

from this department spent 10 days in the epidemic areas from 4th to 13th February. Some 150,000 mosquitoes and 473 bird bloods were collected from water-bird habitats close to localities where confirmed or suspected cases had been reported, and from farm buildings, chicken houses, etc.

MVE virus was recovered from the brains of fatal cases during the last major epidemic in 1951 (and in 1917, 1918 and 1925), but, as with RRV, isolations from mosquitoes have been only from tropical areas in north Queensland and New Guinea, and those were not associated with epidemics. It is hoped that sufficient isolates will be made from the mosquito collections to incriminate the major epidemic vectors (as postulated by Dr. Bill Reeves in 1954), and that evidence from the bird bloods and sera will elucidate the theoretical primary cycles. However it must be admitted that the collections are somewhat biased towards *Culex annulirostris* and water-birds, (29 spp. of water-birds, 7 spp. of land birds).

MVE virus activity was detected in N.S.W. in 1956 and 1971, and in the latter year there were concurrent cases of horse encephalitis. The cause of the latter was not elucidated; similar clinical cases produced rises in antibody titres to MVE and/or RR virus. Horse encephalitis has been reported again this year and another effort is being made to detect the causative agent.

At the time of writing there have been 16 confirmed cases of MVE in humans and 3 deaths, 2 of which have been confirmed. These have occurred over a very wide area of the Murrumbidgee and Murray Rivers, although associated mostly with the latter. The outbreak has not reached the proportions of 1951 (45 cases, 19 deaths), but the deaths are the first in 23 years in the area from which the virus name is derived.

(Ian Marshall)

| Collection site | Species & Pool Size |
| --- | --- |
| Warren | Culex annulirostris (50) |
| Brigalow | " " (50) |
| | " " (50) |
| | " " (50) |
| | " " (50) |
| | " " (50) |
| | " " (50) |
| | Mansonia uniformis (16) |
| | Culex sp. (1) |
| | Culex annulirostris (50) |
| | " " (50) |
| | " " (50) |
| | " " (50) |
| Wanaaring | Anopheles annulipes (50) |
| Wilcannia | " " (50) |
| Menindee | Culex annulirostris (50) |
| Wentworth | - |
| Hay | - |
| Barren Box | - |

| Group | Virus | Total Mosquitoes |
|---|---|---|
| ? | ?* | 306 |
| A | Ross River | |
| " | " | |
| " | " | |
| " | " | |
| " | " | |
| " | " | |
| " | " | |
| " | " | |
| A | Sindbis | |
| B | Kokobera | |
| ? | MB69[†] | |
| ? | ? | 8257 |
| Mapputta | Trubanaman | 2753 |
| " | Mapputta | 176 |
| ? | ? | 310 |
| - | - | |
| - | - | 880 |
| - | - | 824 |

REPORT FROM THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF WESTERN AUSTRALIA, PERTH, W.A., AUSTRALIA

Over 50% of Australia's surface water resources are located above the 20th parallel of latitude. The Ord River Project (Stanley, 1972, Alpers *et al.*, 1972) is the first large man-made lake in tropical Australia and is considered to be a pilot scheme for the development and utilisation of the vast water resources of northern Australia. The sudden creation of this huge area of water (Fig. 1) and its associated irrigation areas has been the inducement for long term ecological studies of infections likely to affect man and other vertebrates in this area. This report refers only to recent and renewed investigations of arboviruses since our initial studies reporting the endemicity in this area of Group A and Group B viruses more than a decade ago (Stanley and Choo, 1961).

The problem is basically one of the evolution of arboviruses in South-East Asia (Fig. 2) and it is anticipated that progress reports in three areas will regularly become available--viz., (1) mosquito programme, (2) virus isolation and identification, and (3) serological epidemiology. Adequate interpretation of the data summarized below must await further studies in adjacent areas in other seasons. The man-made lake is now about 1/3rd full.

1. Mosquito fauna of the Ord River area.

Two field trips were made in 1972 to coincide with seasonal changes. The greatest number, both in concentration and species diversity, were found close to human habitation at Kununurra (Fig. 1.). The dam itself (Lage Argyle) had low numbers during both trips, but this may well change once the dam has completely filled and the system stabilized at a new level. *Culex annulirostris* was the dominant species. Table 1 shows a general summary of the mosquitoes in the area.

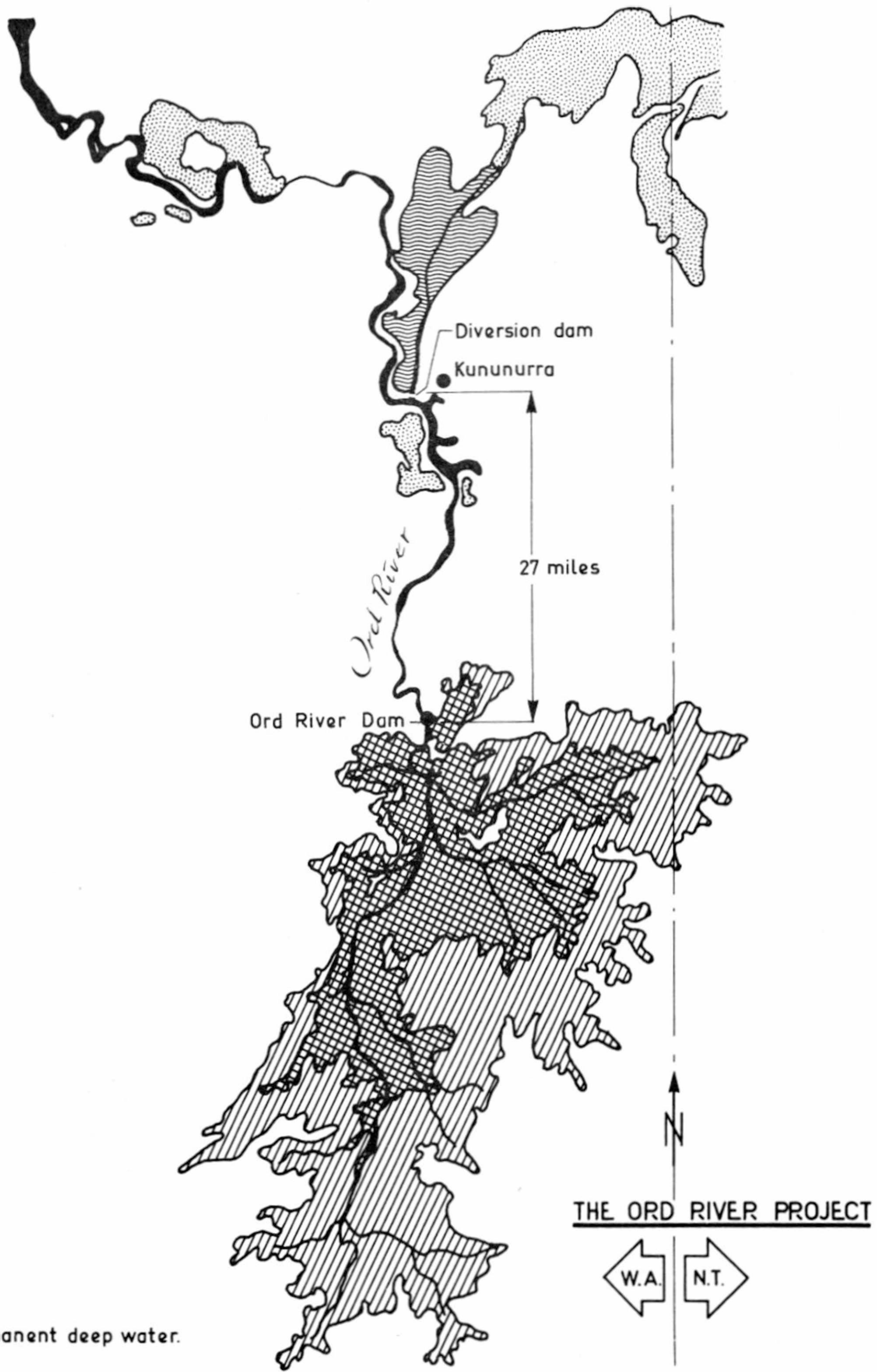
2. Virus isolation from mosquitoes.

Isolation attempts were positive only from pools of *Culex annulirostris* collected near Kununurra in May/June 1972. All isolations were made in newborn mice. Four types of virus were isolated and presumptive identification confirmed through the courtesy of the Queensland Institute for Medical Research as seen in Table 2.

3. Serological epidemiology.

H-I and neutralization tests for viruses are being continuously performed on (1) human sera for antibody conversion rates, (2) avian sera including sentinel chickens, (3) bats, (4) cattle and (5) horses. Initial studies with MVE, Ross River and Sindbis antigens confirm the endemicity of MVE and Ross River viruses. Results will be reported as they become available.

(N.F. Stanley, H. Peterson, P. Liehne, S. Lievers)


Diversion dam
Kununurra
Ord River
27 miles
Ord River Dam
THE ORD RIVER PROJECT
W.A.
N.T.
N
Permanent deep water.
Shallow stagnant water.
Irrigation areas.
Future irrigation areas.

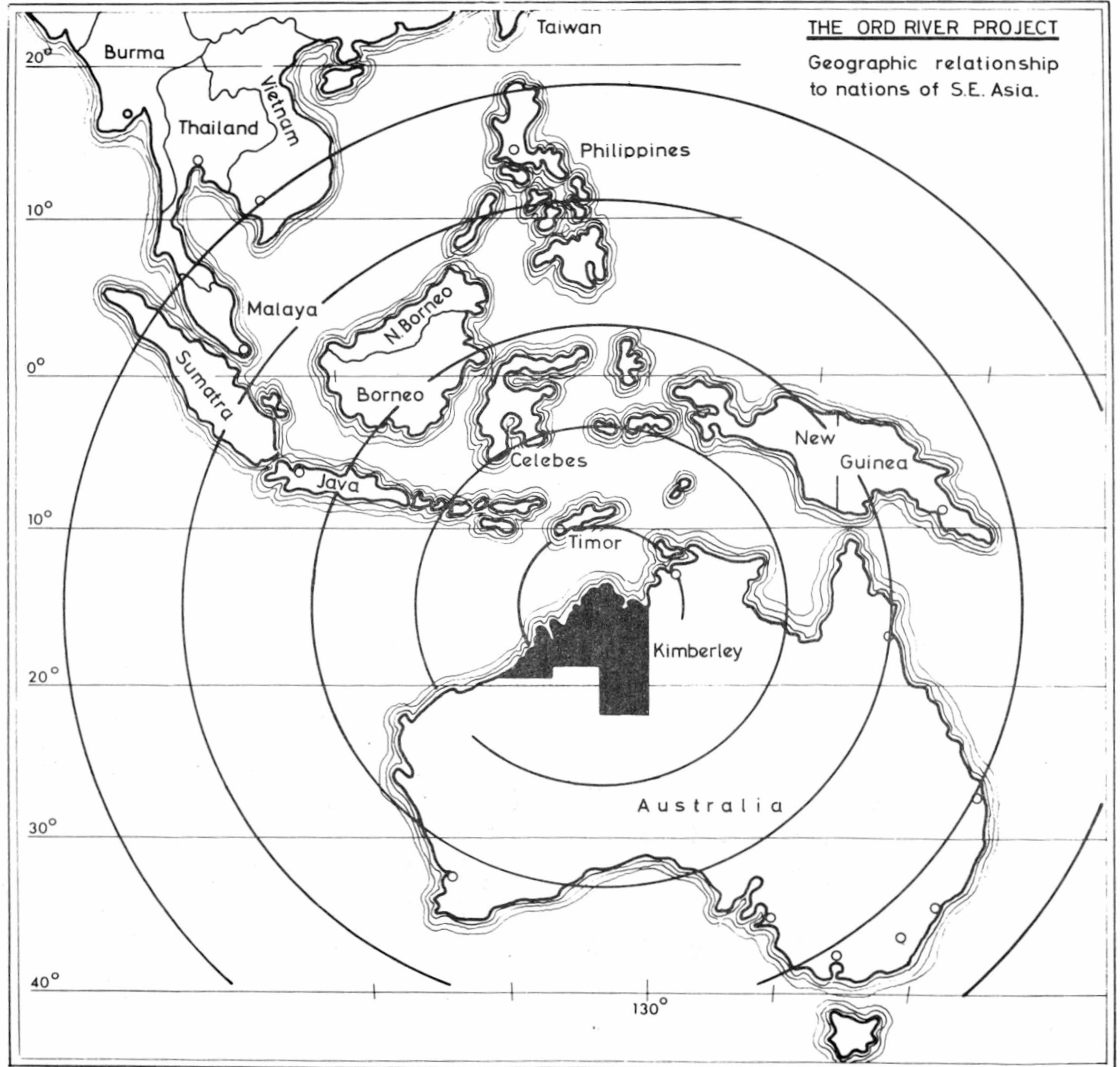

THE ORD RIVER PROJECT
Geographic relationship
to nations of S.E. Asia.
Taiwan
Burma
Thailand
Vietnam
Philippines
Malaya
N. Borneo
Sumatra
Borneo
Celebes
New
Guinea
Java
Timor
Kimberley
Australia
20°
10°
0°
10°
20°
30°
40°
130°

Table 1. CHECKLIST OF MOSQUITOES OF THE ORD

Key to Notation:

A = Adult
L = Larvae
* = form indicated was common
K.R.S. = Kununurra

| Species | Records in Literature | March/May 1972 | November 1972/ January 1973 |
|---|---|---|---|
| Aedes (Finlaya) notoscriptus | | | A,L Kununurra |
| Aedes (Macleaya) tremulus | | A Argyle<br>Kununurra<br>Point Springs | |
| Aedes (Mucidus) alternans | | | A,L Kununurra |
| Aedes (Ochlerotatus) normanensis | | | A Wyndham<br>Parry's Ck. |
| Aedes (Ochlerotatus) vigilax | K.R.S.<br>Wyndham | | A* Wyndham |
| Aedes (Stegomyia) katherinensis | K.R.S.<br>Ivanhoe<br>Wyndham | | L Kununurra |
| Aedomyia catasticta | | A Kununurra | A* Kununurra |
| Anopheles (Cellia) annulipes complex | K.R.S. | A* Wyndham<br>Kununurra<br>L* Argyle | A*,L* Kununurra |
| Anopheles (Cellia) amictus | Argyle<br>K.R.S.<br>Wyndham | A Wyndham<br>Kununurra<br>L Argyle | A,L Kununurra |
| Anopheles (Cellia) hilli | Argyle<br>K.R.S.<br>Wyndham | | |
| Anopheles (Cellia) novoguinensis | K.R.S.<br>Wyndham | | |
| Culex (Culex) annulirostris | K.R.S.<br>Wyndham | A* Wyndham<br>Kununurra<br>L* Argyle | A* Wyndham<br>Kununurra<br>L* Argyle |
| Culex (Culex) australicus | | A Wyndham | A Wyndham |
| Culex (Culex) bitaeniorhynchus | | | A Kununurra |
| Culex (Culex) crinicaudia | K.R.S. | | |
| Culex (Culex) fatigans | K.R.S.<br>Wyndham | A,L Kununurra | A,L Kununurra |
| Culex (Culex) sitiens | | L Kununurra | |
| Culex (Culex) squamosus | | A,L Kununurra | |
| Culex (Culex) starkiae | K.R.S.<br>Wyndham | | |
| Culex (Culex) vicinus | | A Kununurra | |
| Culex (Culiciomyia) | | A Argyle<br>Kununurra | |
| Tripteroides punctolateralis | | A Argyle<br>Kununurra | A Kununurra<br>L Argyle |

Table 2

Provisional local nomenclature of Ord River viruses

| | | |
|---|---|---|
| MVE type | MSL 10 | OR 1 |
| | MSL 11 | OR 2 |
| | MSL 21 | OR 3 |
| KUNJIN type | MSL 13 | OR 4 |
| WONGAL type | MSL 12 | OR 5 |
| SINDBIS type | MSL 14 | OR 6 |

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

## Chikungunya Virus haemagglutination: study in different pH and erythrocytes of different species of animals.

Haemagglutination (HA) reaction of an African strain of Chikungunya (AC) and a strain of the same virus isolated in Calcutta (C63611) was tested in different pH and using erythrocytes of different species of animals, to determine the optimum pH requirement and preference of erythrocytes.

HA of the AC strain was observed between pH 6.2 and 6.4 while that of the C63611 strain was specifically observed at pH 6.2.

Regarding erythrocytes the following table shows the results:

| | HA titre | |
|---|---|---|
| Erythrocytes | C63611 | AC |
| Goose | 1280 | 640 |
| Fowl | 320 | 80 |
| Cat | 1280 | 320 |
| Mouse | 20 | 20 |
| Sheep | 640 | 160 |
| Monkey | 2560 | 320 |
| Rabbit | 20 | 20 |
| Guinea pig | <10 | <10 |

(S.K. Chakravarti, K.K. Mukherjee, J.K. Sarkar.)

## COMMENTS FROM THE EDITOR--NOTICE OF NEXT DEADLINE

It will be obvious to all who peruse this issue of the Info-Exchange that the standards of the reports submitted remain at a highly significant level. I have also been generally pleased with the quality of the tabulated and graphic material submitted, which has permitted clear photographic reproduction.

The deadline for the next issue (Number 27) is September 1, 1974. Please mark your calendar and try your best to meet this deadline. If the deadline is closely observed, the issue can be in your hands by October 1.

The address is as follows: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