June, 1992

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PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE:

To exchange information on a timely basis. The recipients are those who study various aspects of arboviruses. The Exchange contains preliminary reports, summaries, observations, and comments submitted voluntarily by qualified agencies and individual investigators. The appearance in the Exchange of any information, data, opinions, or views does not constitute formal publication and should not be referred to in "Reference" sections of papers or included in lists of publications. The Exchange is not a "peer reviewed" publication; in fact, it is not a publication at all. Any reference to or quotation of any part of the Exchange must be authorized directly by the agency or person submitting the text.

GUIDELINES FOR SUBMITTING REPORTS

We want to keep this mechanism timely and viable. Therefore, submit only recent news and summaries of your work. **PLEASE** limit the submission to 1 or a very few sheets (21.59 cm x 27.94 cm = 8.5 x 11 inches) plus a table or two; condense as much as you can (**single space the text**); **do not** staple pages together; **do not** number pages.

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NOTE: As noted in "PURPOSE OF THE ARBOVIRUS INFORMATION EXCHANGE", which is found on the front page of each issue, you are encouraged to submit a brief summary of your work. The summary need not be in manuscript style, the results do not have to be definitive, you need not include tables (unless you want to). This is not a peer-reviewed publication. The intent is to communicate among ourselves and to let each other know what we are doing.

The next issue will likely be mailed December 1, 1992 (probable deadline for submissions: November 15, 1992). There is nothing that requires you to wait until the last minute. If you have something to communicate in July, August, September, or October, please send it. Also, there is nothing that prevents you from submitting a report to every issue. There are no pages charges either but, then again, this is not a publication.

PLEASE !!!

Follow the directions for submitting reports. Double-spaced pages take twice as much space as single-spaced pages. Do not double-space or number pages. Single-space them and leave them unnumbered. **Do not staple pages together.**

Charles H. Calisher, Ph.D.
DVBID/NCID/CDC
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National Center for Infectious Diseases
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P.O. Box 2087, Foothills Campus
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May 21, 1992

Arthropod-Borne Virus
Information Exchange Recipients
Worldwide

Dear AIE Recipient:

As Dr. Calisher notes in his Editor's Comments of this issue of the Arthropod-Borne Virus Information Exchange, he will be leaving CDC June 1 after a long and distinguished career. His departure will mark the end of an era at CDC, and we most definitely will miss him and his expertise. We wish him the best in his new life in retirement.

We agree with Dr. Calisher that "this is important stuff after all." My guess is that most of us never had any doubts. For reinforcement of our belief that arbovirology is important, all we need to do is observe the numerous new and resurgent diseases that have become major public health problems in the past 10 years, many of which are arboviruses.

The purpose of this letter is to let you know that we at CDC feel that the AIE is an important mechanism to continue communication and information exchange between arbovirologists and other scientists working on vector-borne infectious diseases. I want to take this opportunity to emphasize that the Arthropod-Borne Virus Information Exchange will continue and urge all of you to continue to submit reports.

Although we do not yet have a new editor identified, the AIE will continue to be assembled and sent out from this Division as it has for the past 25 years. Until a new editor can be found, reports and correspondence can be sent to me.

Finally, I want to take this opportunity to thank all of you for your support of AIE for all of these years. We look forward to working with you in the future.

Sincerely yours,

Duane J. Gubler

Duane J. Gubler, Sc.D.
Director

cc: Dr. Joel M. Dalrymple
Chairperson, ACAV

Editor's comments

After 27 years and 48 days, I am leaving CDC. At about the time this issue goes out, so do I. Therefore this is my last as Editor of the AIE, an important means of communication between arbovirologists. Please accept my sincere thanks for your cooperation. You made this job a pleasure.

On another and more pleasant note, I am more convinced than ever that the American Committee on Arthropod-borne Viruses (ACAV) and its Subcommittee on Inter-Relationships Among Catalogued Arboviruses (SIRACA) has been on the right track all along. Formerly named the Subcommittee on Immunological Relationships Among Catalogued Arboviruses, for nearly 30 years SIRACA has examined the registration cards and looked for duplicates, tested to determine antigenic relations, and tried to place arboviruses in some semblance of order. Often its members worked in a vacuum, with some non-SIRACAns expressing the opinion that this really does not matter very much when disease or funding or other important aspects are considered.

Recently, the International Committee on Taxonomy of Viruses sponsored a virus word list book to be published by Williams & Wilkins. Half way through the writing of the book it became apparent that virus names are a disaster of inconsistencies. Whether to italicize, whether to capitalize, whether hundreds of recognized bacterial viruses are really different from each other or are simply pet names (C_2, 3T+, 44RR2.8t, and α are not instructive), what to do with viruses that belong in groups but for which no one has the data and temerity to suggest family and genus placement, whether to use hyphens between virus names and numbers, etc. all may seem beyond trivial, until one tries to create a data base and must deal with this lack of uniformity. Then there is the question of acronyms, for which presently there are duplicates, triplicates, and quadruplicates beyond belief. Journal editors must be cursing virologists for the problems we have presented simply because we have not attended to details.

Arbovirology still has people who use the term "dengue virus", when they mean a particular virus species that causes dengue, or use the term "eastern subtype" (or western subtype, or eastern variant, etc.) of HFRS virus, when they mean Hantaan or Puumala virus, or use the term "bluetongue virus", when they mean a particular virus of the bluetongue serogroup. These folks are still with us, Neanderthal-like and causing confusion. Nevertheless, because of the efforts of SIRACA and others, vertebrate virology is far ahead of bacterial, fungal, algal, and plant virologists when it comes to taxonomy and classification. All that SIRACA has done will have to be done by the other interest groups if they are to get with the program. They have not cared, therefore they have not understood, therefore they are out of touch.

Congratulations to the founding fathers of ACAV for their foresight. As it happens, this is important stuff after all.

Obituary

It is with great sadness that I report the death of Dr. Jelka Vesenjak in Zagreb, Croatia, February 21, 1992. Jelka was an Honorary Member (1980) of the American Society for Tropical Medicine and Hygiene and a long-time collaborator with C.D.C. on studies of tick-borne encephalitis (TBE) and other arboviral diseases.

In the late 1930's, Jelka joined the fight against fascism in Slovenia, the place of her birth, became the personal physician to Josip Broz (Marshall Tito), and was awarded many military and civil honors. After numerous harrowing experiences during the Second World War and having come close to death from typhus and from enemy bullets, Jelka embarked on a career in epidemiologic and laboratory research in Slovenia and Croatia. Among other accomplishments, and under somewhat less than optimal conditions, she headed a team that identified a focus of TBE in Croatia, isolated the etiologic agent (Central European Encephalitis virus) from humans and from ticks, showed that movement of sheep to southern pasturages allowed the distribution of infected ticks from north to south, was first to describe the presence of hemorrhagic fever with renal syndrome in Croatia, was first to isolate Bhanja virus in Yugoslavia and first to describe human infections with that virus, and was responsible for seminal studies of typhus and Q fever on the Adriatic islands of Dalmatia. Her laboratory studies of bacterial, parasitic, and viral infections never obscured her love for clinical medicine and her love for people.

Jelka was appointed Director of the (Andrija Stampar) School of Public Health of Croatia. She was a founder or member of numerous virology and microbiology societies in Zagreb, in Croatia, and throughout Yugoslavia and Europe. She tirelessly organized symposia, published scientific papers and edited books, traveled widely to meetings and for personal satisfaction, led delegations of tourists, guided graduate students, and was inquiring to the end.

Because of her experiences during the war and the many close brushes she had with death, she had a joy for living that was remarkable. Jelka loved children, animals, evenings drinking wine with working people, telling jokes, talking politics and philosophy, the naive paintings of Yugoslavia-- everything; she was first up in the morning and last to bed at night.

My personal relation with Jelka began with a P.L. 480 project to study TBE in Yugoslavia; Jelka was the Project Director and I was the Project Officer. Usually, I went along for the ride because she did not need very much assistance and I did not know anything anyway. In the 20 years of our collaboration I learned as much about epidemiology, customs, wine, art, music, people, and life in general as I have from anyone. Listening to her relate her war-time experiences (spending three days jammed into the chimney of a farm house to evade Nazi soldiers, providing medical care for American paratroopers, being told that her brother had been executed, contracting typhus and being left in the woods to die, resuscitating a child who had drowned), going to the symphony with her in Zagreb,

spending an afternoon talking to members of a rural family about TBE, sitting at the edge of the woods looking for ticks, being cared for by her when we were on an isolated island in the Adriatic and I had influenza, fishing illegally with her friends on the coast, touring the Dioclesian Palace in Split, accidentally wandering across the Albanian border, or dancing all night and drinking too much. It was quite an experience for me. She was a long-time member of the Communist Party (I believe she retained her membership to take advantage of discounts in public transportation) and avid socialist. So it was always a surprise when Jelka found enough Jews (no matter what time of year, what time of day, or where we were) to make a quorum for prayers when I was in Yugoslavia and should have been at home.

Jelka was goal-oriented but always took the time to take the edge off a potentially difficult situation, to take time to laugh at herself or at a political joke, or at anyone who seemed self-important, or to look for a little better quality wine. She knew at least someone and was welcome everywhere. When I was with her we never seemed to pay for food, drink, or transportation, although we ranged far and wide. Much of this hospitality was typically Croatian but some of it was gratitude for her medical and other war-time assistance. Through her intercession, the Calishers were fortunate to have a young visitor from Yugoslavia in our home for a year.

Jelka's heart ached at the pain endured by the once and former country of Yugoslavia, a country she had worked so hard for. I am certain a local physician pronounced a definitive and pathophysiologic reason for her death. However, whereas she lived to a reasonable age, I have no doubt that Jelka's death was in some part due to the current situation in her beloved country. Jelka Vesenjak was a remarkable human being, a scientist, a woman, and a friend to decent people who passed her way. The world is diminished by her absence.

Jelka is survived by her devoted and loving husband, Dr. Franjo Hirjan (Jabukovac 27 [Tuscanac], 41 000 Zagreb), retired former Justice of the Supreme Court of Croatia. I am certain that Franjo would be pleased to hear from Jelka's friends and admirers.

Stedman's/ ICTV Virus Words

This alphabetical compilation of over 2,500 virus names allows users to ascertain the correct spelling of all validly published virus names, identify their taxonomic status, determine the validity of a name, and recognize accepted synonyms. Also included are the complete taxonomy of virus, accepted abbreviations of virus names, and diagrams of virus families and groups according to their given major host.

1992 / 304 pages / #7956-5 / $28.00

BULLETIN DE COMMANDE

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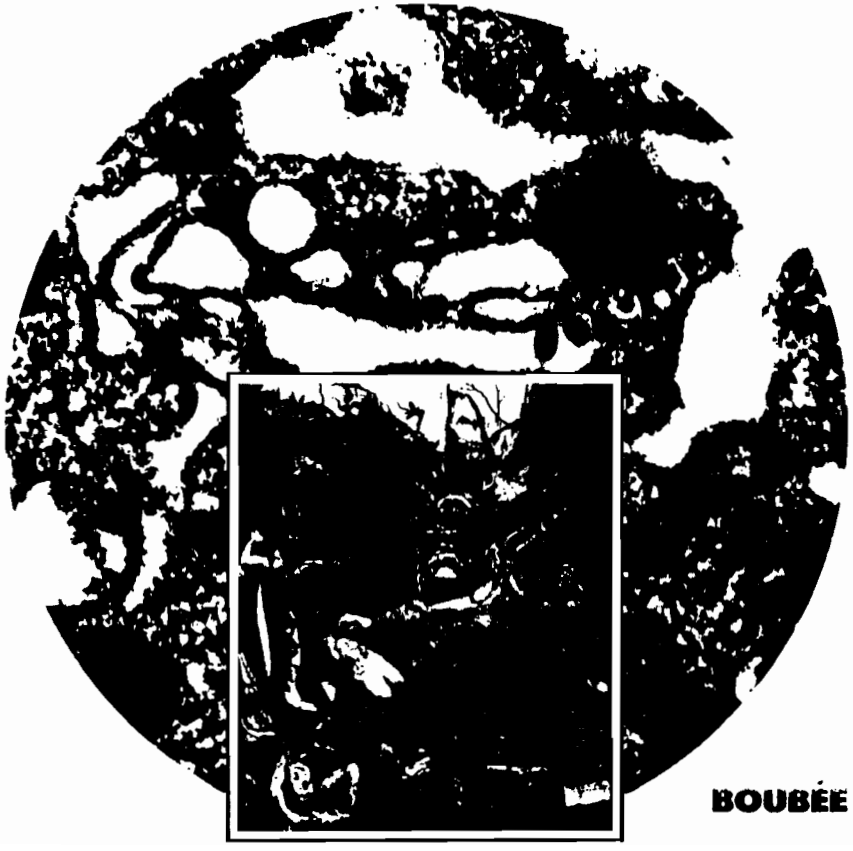
CLAUDE CHASTEL

HISTOIRE DES VIRUS

DE LA VARIOLE AU SIDA



BOUBÉE

Les virus ont été découverts il y a un siècle par un jeune étudiant russe, D.I. Ivanowski, qui indentifia en 1892 l'agent étiologique d'une maladie du tabac, la mosaïque. Depuis, ils n'ont cessé de susciter un intérêt considérable auprès des biologistes, car leur nature est restée longtemps mystérieuse. D'abord définis par des caractères négatifs, l'impossibilité de cultiver dans les milieux artificiels de la bactériologie et l'invisibilité au microscope, les virus filtrants ou ultravirus sont devenus plus simplement les virus lorsqu'ils ont émergé, dans les années 1930, d'autres concepts tout aussi flous à l'époque, les molécules, diastases, inclusions, gènes, etc. Cependant, ce n'est qu'à partir des années 1950, avec les progrès de la génétique, de la biochimie, des cultures cellulaires et du microscope électronique, que l'on a pu enfin comprendre leur nature et la façon dont ils infectent les cellules vivantes: ils se comportent en parasites absolus, au niveau génétique (Luria, 1945), perturbant en profondeur les équilibres fragiles du monde vivant et participant, avec d'autres éléments transposables, à l'Evolution.

Responsables de fléaux aussi redoutables que la variole et la fièvre jaune, et maintenant le SIDA, leur histoire se confond souvent avec celle de l'humanité. Mais ils peuvent aussi avoir un rôle plus incidieux comme le font les virus lents ou les virus oncogènes.

Cet ouvrage est destiné aux historiens des Sciences, aux enseignants et aux étudiants en biologie animale et végétale, en médecine, pharmacie, art dentaire et art vétérinaire, ainsi qu'à tous ceux, moins spécialisés, qui sont curieux des mystères de la vie.

L'auteur, médecin, épidémiologiste et virologiste, est professeur de microbiologie à la Faculté de Médecine de Brest, Université de Bretagne occidentale. Ayant terminé ses études de médecine au moment où la virologie commençait à prendre son essor, travaillant depuis plus de 30 ans sur les virus, il a pu mesurer les immenses progrès de nos connaissances dans ce domaine.

PARUTION FEVRIER 1992

CLAUDE CHASTEL
Professeur de microbiologie à la Faculté de Médecine de Brest.

HISTOIRE DES VIRUS DE LA VARIOLE AU SIDA

Publié avec le concours de l'Université de Bretagne occidentale.
Illustré de 45 figures dans le texte. Un volume 16 × 24 cm d'environ 420 pages, broché sous jaquette en couleurs.
Prix: 320 F

EVIDENCE SUGGESTING A ROLE FOR GLOSSY IBIS IN THE EPIDEMIOLOGY OF EEE[1]

WAYNE J. CRANS[2], DONALD F. CACCAMISE[2] AND JAMES R. MCNELLY[3]

INTRODUCTION

A cooperative research study involving, Rutgers University, the Cape May County Mosquito Control Commission and the N.J. Department of Fish, Game & Wildlife, was initiated in 1988 to examine the role of salt marsh wading birds in the epidemiology of eastern equine encephalitis virus (EEE), a debilitating disease of humans that is carried by wild birds and transmitted by mosquitoes in coastal areas of southern New Jersey. During the first year of the study, 213 estuarine birds were captured by cannon net, bled from the wing vein and tested for antibody to EEE virus by the Centers for Disease Control (CDC) in Fort Collins, CO. Bloods were also screened for Highlands J virus (HJ), a benign virus that is transmitted to birds by the same mosquito vectors that perpetuate EEE. Data revealed that Snowy Egrets, the most common birds on the New Jersey marshes, had low levels of antibodies to both viruses, indicating infrequent contact with the mosquito vectors. Glossy Ibis showed high levels of infection to both viruses but the sample sizes for these birds was low during the first year of the study.

Since 1988, a concerted effort has been made to capture both Snowy Egrets and Glossy Ibis to obtain a more complete blood profile on the antibody history of these salt marsh birds. Analysis of numerous samples have confirmed that Glossy Ibis make repeated contact with both EEE and HJ virus; Snowy Egrets appear to avoid contact with either virus. Table 1 lists the results of the 1019 samples we have taken to date by species and age class. The data indicate that Glossy Ibis (regardless of age) are far more apt to make contact with the mosquitoes that transmit the encephalitis viruses.

Research has focused on behavioral mechanisms that may account for repeated contact with the mosquito vectors of EEE or avoidance of the mosquitoes that carry the disease. We have employed banding, radio telemetry and color-marking in addition to the blood sampling that marked the initiation of the research. This paper interprets the information we have accumulated to date and describes the goals of the project over the next several years.

LOCATION OF ROOKERIES IN SOUTHERN NEW JERSEY

Beginning in 1989, a Spring helicopter survey has been undertaken each year to locate the nesting sites of Glossy Ibis and Snowy Egrets in the southern portion of New Jersey. The major rookeries have been used to make comparisons on the juvenile behavior of each species from hatching to dispersal during the encephalitis season. Data indicate that all of the wading birds nest on the Atlantic side of the state in areas that are virtually mosquito free. No rookeries have been found in areas where EEE is known to be enzootic. The largest rookeries have been located at Stone Harbor and Townsends Inlet in southern Cape May County. Smaller rookeries have been located near North Wildwood, in southern Cape May County, Ocean City, in northern Cape May County and at Margate in Atlantic County. Mosquito trapping data confirm that the nesting sites are virtually mosquito free. Table 2 compares the mean number of mosquitoes trapped per night in a pigeon-baited CDC trap at the Stone Harbor rookery (where EEE is absent) vs. a Delaware Bay roosting site (where EEE cycles every year). The data clearly show that contact with mosquitoes is minimal until the birds disperse to Delaware Bay.

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[1] New Jersey Agricultural Experiment Station Publication No. E-40101-04-91 supported by State Funds and a Grant from the Marine Sciences Consortium.

[2] Department of Entomology, P.O. Box 231, Cook College, New Brunswick, NJ 08903.

[3] Cape May County Mosquito Extermination Commission, P.O. Box 66, Cape May Courthouse, NJ 08210.

Table 1. The Antibody Profile of Glossy Ibis and Snowy Egrets Captured in Cape May County from 1988 - 1991.

SPECIES	AGE	NO. BLED	ANTIBODY POSITIVE *	
			No.	%
GLOSSY IBIS	Nestling (L)	139	6	4.3
	Hatching Year (HY)	174	22	12.6
	Adult (AHY)	219	47	21.6
SNOWY EGRET	Nestling (L)	55	0	0
	Hatching Year (HY)	266	7	2.6
	Adult (AHY)	166	8	4.8

* Positive to EEE, HJ or both Viruses

Table 2. Mosquito Populations in Atlantic Coast Rookeries vs. Delaware Bay Roosting Sites in 1990.

HABITAT	DATES TRAPPED	TOTAL MOSQUITOES	MOSQ. PER TRAP NIGHT
Stone Harbor Rookery	5/23-7/19	8	0.3
Cedar Swamp Roosting Site	7/5-9/20	11,470	318.6

In 1990, large numbers of the Cedar Swamp mosquito, *Culiseta melanura*, were trapped near Glossy Ibis roosting areas and tested for EEE virus. *Culiseta melanura* is recognized as the main vector of EEE to birds and has been classically used as the indicator for EEE activity in nature. Table 3 lists the numbers tested by month and the results of the virus isolation tests. The data show that EEE was cycling in wild birds and *Cs. melanura* in July and August. By September, virus activity had all but terminated.

Table 3. Virus Isolations from *Cs. melanura* in the Dennis Creek Study Site during 1990.

MONTH	No. Tested	No. Pools	EEE Positive	MFIR*
JULY	383	13	3	7.83
AUGUST	929	26	3	3.23
SEPTEMBER	447	13	0	0.00

* Isolations per thousand specimens tested

DISPERSAL PATTERNS FROM THE NESTING SITES

A concerted banding effort was initiated at each of the rookeries to mark nestlings from Cape May and Atlantic counties with aluminum leg bands to assure recognition of local birds in future encounters. A total of 23,651 wading birds have been banded to date with emphasis on the 2 target species. Table 4 lists the number of birds banded by year.

Table 4. Salt Marsh Wading Birds Banded in Southern New Jersey from 1989 to 1991.

SPECIES	1989	1990	1991	TOTAL
SNOWY EGRET	3302	4589	3176	11,067
GLOSSY IBIS	1276	4339	4943	10,558
OTHER *	584	1074	368	2,026
TOTAL	5162	10,029	8487	23,651

* Black-Crowned Night Heron, Great Egret, Little Blue Heron, Tricolored Heron

Over the 3 year period, twelve (12) Glossy Ibis immatures and ten (10) Snowy Egret immatures were fitted with radio transmitters to monitor dispersal patterns from the nesting sites after the birds left the rookeries. In addition, approximately 250 fledgling birds of each species were color marked in 1990 and 1991 to monitor dispersal from the rookeries on the Atlantic coast. Glossy Ibis were color marked by bleaching the dark feathers to a pale blond color with Clairol hair stripper. Snowy Egrets were color marked with Black Clairol dye in 1990 and with "Rhodamine Red" dye in 1991. The black dye faded quickly and was not discernable by the time the Egrets dispersed from the colonies. The "Rhodamine Red" faded to a bright pink over time and was clearly discernable for the duration of the 1991 season.

Data from the radio tagging, banding and color marking studies show that Glossy Ibis fledglings leave the rookeries on the Atlantic coast at the end of June and travel directly to the Delaware Bay marshes where EEE is known to cycle in passerine birds and mosquitoes. Radio telemetry data points showed that the young Ibis did not return to their nests on the Atlantic coast but dispersed widely along the Delaware Bay marshes from Dennisville, in Cape May County, to Dividing Creek, in Cumberland County. Hundreds of color marked Ibis have been sited each year and the
residence along Delaware Bay. The majority of juvenile Ibis on the Delaware Bay marshes are fledged in southern Cape May County but young Ibis from Ocean City and Margate have been detected. Preliminary studies on diet suggest that the young Ibis depend on the rich supply of Horseshoe Crab eggs that accumulate along the banks of the tidal creeks. This large food reserve appears to attract the birds in large numbers.

The Snowy Egret fledglings remained close to their nest sites on the Atlantic coast during the months of July and August. Color marked birds frequently foraged several miles from the colony but returned to their nests each day. When the birds did finally leave the colonies they dispersed widely. Many traveled North along the Atlantic coast, others traveled North along Delaware Bay and sightings from Maryland and Virginia suggest that some disperse to the South. Preliminary studies on diet show that the Egrets are primarily fish eaters. Foraging patterns show that fish are abundant close to the Atlantic coast colonies and the Delaware Bay marshes show no particular attraction to these birds until much later in the season. Cannon net recaptures of banded birds have provided another parameter to document the dispersal patterns. During the months of July and August, numerous Glossy Ibis that were banded on the Atlantic coast have been captured on Delaware Bay. Juvenile Snowy Egrets that were banded on the Atlantic coast are not recaptured until late September and October when the birds congregate for their southern migration.

DAILY MOVEMENTS AFTER DISPERSAL

Over the 3 year period of this study, fourteen (14) juvenile Glossy Ibis have been captured by cannon net along Delaware Bay and fitted with radio transmitters to determine daily movement patterns. Data from 1989 showed that the birds roosted for the night in Cedar Swamps where EEE virus was being isolated from *Culiseta melanura*, the main vector among birds. During the day, the juvenile Ibis foraged in salt marsh areas where they would be likely to make contact with *Aedes sollicitans*, the epidemic vector of the disease. The data suggested that Glossy Ibis may be functioning as a transfer reservoir by picking the virus up from *Cs. melanura* in the Cedar Swamps and bringing it to *Ae. sollicitans* for ultimate transfer to humans and horses.

In 1990, radio telemetry data points revealed that the birds sometimes roosted for the night in shallow salt marsh ponds in areas where the epidemic vector occurred in largest numbers. Mosquito traps placed in the roost ponds showed that some *Cs. melanura* were being attracted from the Cedar Swamps to areas where hundreds of birds were congregating for the night. During the day, the birds, again, appeared to be leaving the tidal creeks to forage on the hay farms where contact with *Ae. sollicitans* would be probable. In both years, however, the majority of radio tagged birds moved out of the study area and the data were gathered only from the few tagged birds that took up residency in the study site.

In 1991, a concerted effort was made to capture and radio tag Ibis that would remain in the study area for a more complete picture on daily movements. To achieve this goal, the cannon net was set on a freshwater pond where the birds gathered to drink and bathe (rather than on the tidal creeks where they foraged for food). Three (3) birds were captured and radio tagged and all remained for most of the season. A fourth represented a nestling that had been tagged at Ocean City but took up residency in the Delaware Bay Study site. Residency allowed for daily readings on most birds and multiple readings on many dates. A distinctive mark on 2 of the birds allowed us to document the exact location of most readings.

Each of the birds foraged on the surrounding tidal creeks during the month of July and roosted in salt marsh ponds at night. The roost sites changed frequently but each bird remained loyal to its particular daytime foraging site. Each bird returned to the freshwater pond several times each day. Roosting in Cedar Swamps was not common in 1991 and was only observed when storms forced the birds to seek

cover. In August, the foraging behavior of the birds changed markedly. Instead of foraging along tidal creeks the young Ibis moved onto the open hay meadows where *Ae. sollicitans* were breeding in tremendous numbers. Data from previous years suggested that the Ibis moved onto the hay meadows during August but all observations were made from a distance and exact locations could not be documented. In 1991, the Ibis were observed feeding among piles of decaying Horseshoe Crab carcasses that had breached the dyke of the hay meadow and were rotting in the shallow water of the marsh. The crab carcasses were heavily infested with Blow Fly larvae and the Ibis were, apparently, taking advantage of that food source late in the season after the Horseshoe Crab eggs had been depleted. *Aedes sollicitans* appeared to be feeding heavily on the birds, evidenced by the defensive behaviors exhibited by the Ibis as they fed (scratching, feather ruffling etc.). Numerous blood engorged mosquitoes have been collected from the feeding stations and precipitin tests will be conducted to determine if the blood contained from the mosquitoes is of Wading Bird origin.

MOSQUITO FEEDING SUCCESS WITH CAGED BIRDS

In 1991, Glossy Ibis and Snowy Egret juveniles were captured by cannon net and held overnight for mosquito feeding success trials. On each trial date, two (2) birds were brought into the laboratory late in the afternoon, placed in separate cages within screened cubicles in an insectary and given fresh water and food. The birds were allowed to acclimate for approximately 4-5 hrs. A video camera was used to monitor their behavior during the acclimation period. Just before sunset, 200 *Ae. sollicitans* were introduced into each cubicle. The mosquitoes had 1 hr of daylight, 1/2 hr of simulated dusk, 9 hrs of darkness and 1/2 hr of simulated twilight to feed. Video cameras recorded all phases of the birds' activity during the light phases of the experiment. In the morning, the birds were removed from the cages, offered fresh water and food and then released at their site of capture. All mosquitoes were aspirated from the cubicles and the number that had successfully fed on the birds during the night were calculated.

The mean feeding success rate for *Ae. sollicitans* on Glossy Ibis was 26.8% (9.3-55.9). The success rate on Snowy Egrets was 17.7% (4.4-31.5). Examination of the video tapes indicated that Glossy Ibis exhibited vigorous anti-mosquito behavior but appeared less effective at warding off mosquito attack than the Snowy Egrets. Data also suggested that the older Ibis were more tolerant to mosquitoes, perhaps through the repeated mosquito attacks we observed after they took up residence on the hay meadows late in the season.

CONCLUSIONS TO DATE

The data we have accumulated to date indicate that the nesting season, dispersal patterns and foraging habits of Glossy Ibis are conducive to repeated contact with the mosquito vectors of EEE. The birds enter high risk areas early in the season and remain during the period of greatest enzootic transmission. The birds appear to make contact with the mosquitoes either when they roost for the night or when they forage for food. Roosting in trees provides contact with the epornitic vector, *Cs. melanura*. Roosting in salt marsh ponds provides contact with *Ae. sollicitans*. Leaving the tidal ditches to forage on the salt hay farms on Delaware Bay provides further contact with *Ae. sollicitans* during the critical late summer period when human and equine cases are most often contracted. Since *Ae. sollicitans* will feed on salt marsh wading birds in the laboratory, it is reasonable to assume that they take advantage of the birds in nature. The dispersal patterns and foraging habits of Snowy Egrets appear to provide minimal contact with the mosquito vectors of EEE. These birds do not enter EEE areas until the encephalitis season is all but ended.

Our preliminary information provides an exciting model to explain a necessary link in the transmission of a virus that normally remains benign in a "Wild Bird - Bird Feeding Mosquito" Cycle. to a potentially dangerous "Reservoir Bird - Human Feeding Mosquito" cycle. Since the Glossy Ibis is an introduced species, the model becomes even more exciting, because the Ibis could represent the link that was missing in the transmission of EEE to humans prior to its colonization of the New Jersey Atlantic coast.

The Glossy Ibis was introduced to the western hemisphere at about the turn of the century. Until 1940, the breeding range in the United States was limited to Florida. From 1940 to 1970, Glossy Ibis underwent a rapid range expansion that brought them from Florida to Maine. The first breeding record for New Jersey occurred in 1955. By 1957, the birds had reached the Brigantine Refuge in Ocean County and by 1959, Glossy Ibis were no longer considered rare in New Jersey. In 1968, more than 1000 Ibis were counted in New Jersey and by 1973 that number more than doubled. We have evidence to suggest that the population is still increasing. In 1991, we banded nearly 5000 Glossy Ibis chicks from New Jersey and our cannon net recapture rates suggest that we banded less than 40% of the population.

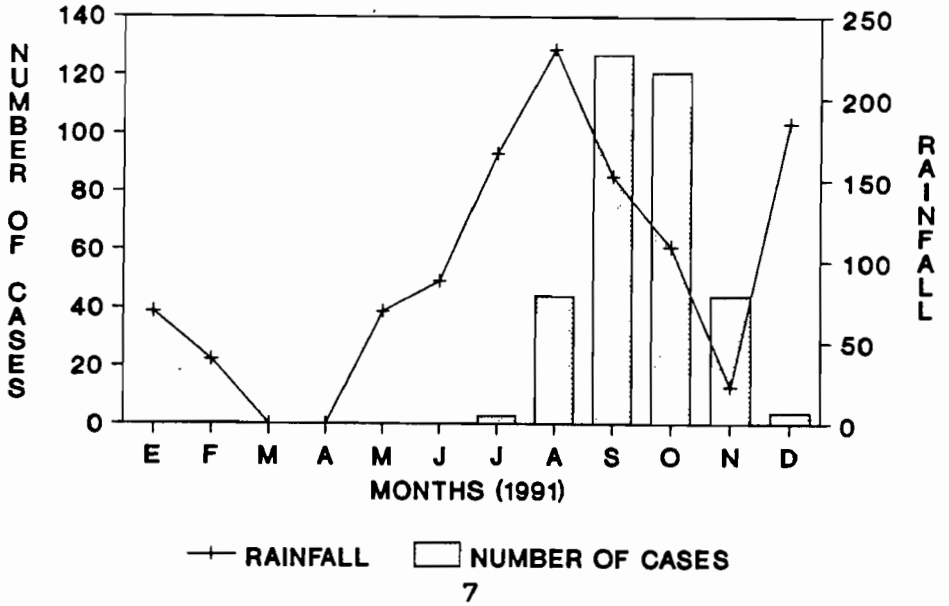
The introduction of the Glossy Ibis to the New Jersey coast closely parallels the sequence of EEE in humans. EEE virus was first isolated from a New Jersey horse in 1933. Although the disease was a new entity, veterinary literature suggests that the virus was being actively transmitted to horses and pheasants since the turn of the century. During the 1950's investigators were detecting high levels of EEE in mosquitoes but the disease was considered a veterinary problem rather that a human health problem. In 1959, New Jersey experienced its first ever epidemic of EEE in humans and the infections were all contracted along the coast from Monmouth to Cumberland Counties. In 1968, New Jersey experienced its second epidemic, with a case distribution that was limited to coastal areas of southern New Jersey. It may have been coincidence that the first cases of human EEE were acquired shortly after Glossy Ibis colonized the New Jersey coast and it may be coincidental that the human cases of EEE in 1959 and 1968 were concentrated in those areas where the birds had established their nesting sites. It is also possible that the Glossy Ibis represents a necessary link in the cycle that was absent in New Jersey prior to 1959.

OUTBREAK OF DENGUE IN MERIDA, YUCATAN, MEXICO IN 1991.

J.A. Farfán, M.A. Loroño, L.F. Flores, E.P. Rosado and L.A. Manzano. Laboratorio de Arbovirus. Centro de Investigaciones Regionales "Dr. Hideyo Noguchi". Universidad Autónoma de Yucatán. Ap. Postal 2-1297, Mérida, Yucatán, México. c.p. 97240.

An outbreak of classical Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) occurred on Mérida, Yucatán from April to November of 1991 (Chart 1). Mérida is the Capital city of the Yucatán State of México, with an altitude of 8 m above sea level. The predominant climate is subtropical. Dengue was reported the first time on 1979. Since that time dengue fever cases have been reported each year. Serotype 1 was identified during the first epidemic. In 1984, it was identified serotype 4 and the first DHF case was reported. On 1991, the laboratory confirmed by serology (HI and Mac-ELISA) 343 cases. Dengue serotypes 2 (5 cases) and 4 (7 cases) were isolated from 12 sera of the cases from Mérida. Fifty three cases were classified as primary infection, 233 as secondary, 57 were not possible to classify (ELISA positive) and 35 were negative (Table 1). Fifty nine (17.2%) of the positive cases had at least one hemorrhagic manifestation. However only one case fulfill the WHO criteria in order to be classified as DHF II. He was a male age 7 who presented high fever, abdominal and muscular pain, prostration, nausea and vomiting. During the fifth day after onset, he had hematemesis. He was hospitalized. Mild hematemesis persisted for three days. Blood test revealed Ht 49 %, platelet count 30,000/mm^3. The HI test on days 8 and 16 were 1:20 and 1:160, respectively. IgM ELISA test was also positive. It was classified as a primary infection. He recovery uneventful.

DENGUE CASES & RAINFALL
MERIDA, YUCATAN, 1991



CASES DATA: LAB. ARBOVIRUS, UADY
RAINFALL DATA: COMISION NAL. AGUA

Positive and negative patients according to serology tests, by age group. Mérida, 1991.

Age group	Primary	Secondary	ELISA +	Negative
0 - 9	6	5	5	2
10 - 19	14	30	11	10
20 - 29	12	54	14	9
30 - 39	9	60	13	3
40 +	12	84	14	11
Total	53	233	57	35

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

DENGUE HAEMORRHAGIC FEVER (DHF) OUTBREAK IN CALCUTTA, INDIA, 1990.

S.K. Chakravarti, K.K. Mukherjee, M.K. Mukherjee, P.N. De, N. Bhattacharjee & M.S. Chakraborty.

A large scale epidemic of Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) was observed in the city of Calcutta, during the months of September to December, 1990. Five strains of dengue viruses (all type 3) could be isolated from febrile cases.

The present report is an account of virological and serological investigation of 151 cases, clinically diagnosed as dengue fever (DF), DHF & DSS; as depicted in table - I.

The hallmark of this epidemic was the major involvement of small children & young adults within 15 yrs. of age group (76.2%). Overall case fatality (5.9%) was also confined among these group. Dengue like symptoms were found among 100 (66.2%) individuals while DHF and DSS among 40 (26.5%) and 11 (7.3%) respectively. Features of meningism were detected in 2 children in the DF group, while in the DSS group, free fluid in abdomen was detected in 2 and pleural effusion in one case (all within 5 yrs. of age).

Clinical laboratory tests carried out amongst DHF/DSS cases revealed increased haematocrit values and thrmbocytopenia in most of the cases.

Five mouse pathogenic agents isolated from the acute phase sera, were all identified as DEN-3, by cross CF test and cross neutralization test in suckling mice. Serotesting by haemagglutination inhibition & complement fixation tests carried out with 55 paired sera, revealed evidence of dengue infection in 33 (60%) and flavivirus group reaction in 17 (30.9%). Serological profile of 6 representative paired sera from cases presenting with DHF/DSS have been shown in table II. Presumptive dengue infection by detecting dengue specific IgM antibody by MAC ELISA could also be established in 34 and flavivirus group reaction in 15 out of 90 single sera.

The present outbreak of DHF is considered to be due to DEN-3. It is interesting to mention that a large scale dengue outbreak in 1983 was recorded to be due to DEN-3 also and a note of caution was expressed of a possible threat of DHF in this area in the near future.

DEN-3 is currently an important emerging strain in some of the South-East Asian countries including India, until a few years back, when DEN-2 played the dominant role. The entry and persistence of DEN-3 in countries with the existing other dengue serotypes, calls for a constant vigil of dengue surveillance system, since the emergence of a new serotype may result in fresh haemorrhagic episodes.

Table - I

Salient features of 151 cases of Dengue & Dengue Haemorrhagic Fever in Calcutta - 1990.

Age in years	Male	Female	Total	No. Hospitalized	Features of Dengue*	Features of DHF	Features of DSS**	No. Death
Upto 5 yrs.	33	20	53	42	33	14	6	3
6 - 10 "	31	14	45	30	25	17	3	4
11- 15 "	12	5	17	3	12	3	2	2
16- 20 "	7	5	12	7	8	4	Nil	Nil
21- 30 "	8	7	15	8	14	1	Nil	Nil
31- 40 "	2	Nil	2	2	1	1	Nil	Nil
40 & above	7	Nil	7	5	7	Nil	Nil	Nil
T o t a l	100 (66.2%)	51 (33.7%)	151	97 (69.5%)	100 (66.2%)	40 (26.5%)	11 (7.3%)	9 (5.9%)

* Meningism in 2 cases

** Ascites in 2 & pleural effusion in 2 cases.

Table - II.

Results of serological tests with six representative paired sera.

Patient No.	Age (Years)	Day of illness	Haemagglutination Inhibition Titre *				Complement Fixation Titre *				Interpretation
			CHIK	DEN-2	JE	WN	CHIK	DEN-2	JE	WN	
1.	4	6	<20	80	20	20	<4	8	<4	<4	Dengue
		41	<20	5120	40	20	<4	256	8	8	
2.	6	4	<20	640	20	20	<4	8	<4	<4	Dengue
		30	<20	20480	20	20	<4	>512	8	8	
3.	7	10	<20	10240	40	40	<4	32	4	4	Dengue
		24	<20	>40960	40	40	<4	512	32	32	
4.	24	6	<20	160	40	40	<4	16	<4	<4	Dengue
		25	<20	>40960	20	40	<4	256	16	4	
5.	12	17	<20	40	20	20	<4	4	<4	<4	Flavivirus Group reaction
		29	<20	160	80	40	<4	16	8	8	
6.	28	5	<20	320	80	40	<4	8	4	4	Flavivirus Group reaction
		28	<20	1280	640	640	<4	64	32	16	

* Expressed as reciprocal of highest dilution of serum, inhibiting haemagglutination or fixing complement with corresponding antigen.

REPORT FROM THE ARBOVIRUS UNIT, NATIONAL INSTITUTE FOR VIROLOGY, SANDRINGHAM, JOHANNESBURG, SOUTH AFRICA

Japanese mosquitoes, including Aedes albopictus, imported in tires into Durban, South Africa.

Immatures of the Asian mosquito *Aedes albopictus* (Skuse) have been collected on 4 occasions in tires landed in Cape Town from Japan (Cornel and Hunt, 1991). It is possible that some of these populations of *Ae. albopictus* might survive Cape Town's Mediterranean winter and become established there, since *Ae. albopictus* is known to survive winters in northern Asia (Reiter, 1984, Hawley *et al.*, 1989). However, we consider that it is much more likely that this predominantly tropical species (Hawley, 1988) would establish itself in subtropical Durban should it escape from the premises of tire importers. Thus we are undertaking surveillance for *Ae. albopictus* and other exotic mosquitoes in Durban situated on our east coast at the premises of the 2 largest importers of tires from Japan.

The first aspect of this surveillance began in November 1991, which was the sampling of each consignment of wet tires as soon as the shipping container was landed and opened up at the tire company. Sampling for immature mosquitoes is undertaken for at least one hour and normally at least 10% of the wet tires are examined.

So far 5 consignments of motor car tires, which included wet tires, were landed between November 21, 1991 and January 16, 1992 and have been sampled. Live immature mosquitoes were collected in 3 of these consignments. In the first, 15 larvae or pupae were found, which included 1 *Aedes aegypti* (Linn.) and 8 *Uranotaenia n. novobscura* Barraud as well as 6 small larvae which died before they were large enough to identify. In the 2nd and 3rd consignments 1 *Ur. n. novobscura* and 1 *Ae. albopictus* were identified respectively.

From the total number of wet tires and the proportion of them found to contain mosquitoes, it was possible to estimate the total number of wet tires in each consignment with mosquitoes. These were 189, 20 and 13. These figures suggest that it is possible that some *Ae. albopictus* could have escaped from the tires and established populations outside the premises of the tire company. The second aspect of our surveillance is aimed at the detection of such populations. This project is still in progress and involves the exposure of bamboo pots as ovitraps within the immediate vicinity of the 2 tire importers. *Ur. n. novobscura* is not medically important but exotic *Ae. aegypti* and more particularly *Ae. albopictus* are dengue

vectors. The latter and possibly the former, would be expected to have a higher vector potential for dengue viruses than the indigenous mosquito species along the Natal coast. It is therefore important that control measures are effective for preventing the escape of exotic mosquitoes from the imported tires lest such mosquitoes become established in Durban. A similar system of surveillance is planned for Cape Town, Johannesburg and Pretoria, other centres where containers with imported used tires are also opened up.

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PETER G. JUPP and ALAN KEMP

Report from the Virus Laboratory
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"A rickettsia-like organism isolated from Ixodes uriae ticks and mimicking virus isolations in suckling mice".

Ixodes (Ceratixodes) uriae ticks were collected on February 19, 1990 from one Macaroni penguin (Endyptes chrysolophus) and one Rockhopper penguin (E. chrysocome) breeding on Mayes Island, Kerguelen Archipelago, French Subantarctic Territories (49° S - 70° E).

Pooled tick homogenates inoculated into suckling mice (sm), yielded two strains (Brest Ar/T 2810 and T 2813) of an apparently identical agent that killed sm in 3 - 5 days and shared some properties with tickborne arboviruses. Thus, they were sensitive to exposition at acidity, diethylether and heating one hour at 60°C.

However, the identification of "Mayes" agent proved more difficult than expected and a number of its properties were not in agreement with those of conventional arboviruses : HA or FC antigens were not obtained after classical sucrose - acetone extraction of infected sm brains nor was antibody produced by mice immunized to produce potent ascitic fluids.

Histological sections of infected sm brains showed minimal alterations whereas neuronal and vascular lesions were prominent in the bulb, spinal cord and liver.

Electron microscopy performed on infected sm brains showed some large particles (up to 200 nm) ressembling arenaviruses, paramyxoviruses, or retroviruses, but no clear cut identification was possible.

Since some Bunyaviridae of the group C (Clarke and Casals Am. J. trop. Med. Hyg., 1958, 7, 561) are able to yield antigens only after liver or serum extraction, these procedures were applied to T 2810 and T 2813.

Antigens with a Fc titer of 1 : 128 were prepared from infected livers and screened using all sera and immune ascitic fluids available in our serotheque including the most recent reactives prepared by J.P. Digoutte, Pasteur Institute Dakar, against 80 African mosquito - borne arboviruses. All CF tests were negative.

Electron microscopy was then performed on infected sm livers and gave the solution : some hepatocytes were filled with large intracytoplasmic inclusions containing pleomorphic rickettsia - or Chlamydia - like organisms, 200 to 400 nm large, with sometimes a double outer membrane and undergoing transverse division.

T 2810 and T 2813 strains were then readily adapted to Mc Coy cells at 30° and 37°C furnishing us an effective IFA system for further antigenic analysis.

Using IFA it was demonstrated that "Mayes" agent is different from :

- Chlamydia psittaci, a pathogen infecting seabirds in the Northern hemisphere ;
- Chlamydia trachomatis ;
- Coxiella burneti, another pathogen infecting seabirds in the Northern hemisphere ;
- Cowdria ruminantium ;
- and Ehrlichia phagocytophila.

The taxonomic position of "Mayes" agent among Rickettsiales or Chlamydiales remains to be clarified, but it seems important for arbovirologists, mainly those working on tick-borne arboviruses, to be aware of the possibility to isolate such an agent in suckling mice. Thus, we propose to systematically include "Mayes" reagents during the identification procedures of unclassified or "new" arboviruses.

(C. Chastel, M. Demazure, O; Chastel, F. Genevois, O. Grulet, M. Odermatt and F. Le Goff).

Rapid Detection of Dengue-2 Viral RNA Using Reversible Target Capture Hybridization

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Current research is focused on developing a reversible target capture (RTC) sandwich hybridization technique for the detection of dengue-2 viral RNA and developing new molecular reagents to accurately identify serotypes and topotypes of dengue viruses. The RTC is a form of sandwich hybridization that utilizes two probes: a poly-A tailed capture probe and a labeled detector probe. Both probes are allowed to hybridize to the target nucleic acid in solution. Following hybridization, the poly A tailed capture probe is used to selectively remove the hybrids by capture on poly T magnetic beads. Following capture and several washes, the hybrids are released from the beads, and subjected to the detection procedure. The capture probe used in developing the RTC for detecting dengue RNA was selected using two main criteria: 1) it must have a G-C content of >50% and 2) it must be adjacent to the 5' end of the RNA being used as the detector probe. The sequence selected occupies bases 7086-7127 on the dengue-2 genome and consists of the following sequence: 5'GTGAGTAGCATCCAATGGCGAGAAGGGGAACTCCGATGTCC3'. This 41 base oligonucleotide has a GC content of 56% and is located two bases upstream from RNA transcript probe KOG-500, which begins at base #7129. Initially, the RTC procedure was performed using conditions of hybridization as described by Morrissey (Anal Biochem 181, 345, 1989), then adapted for dengue detection, by individual optimization of all of the parameters of the procedure. In every procedure, a control reaction (full reaction minus capture probe) was included. The following parameters were tested and optimized individually: hybridization temperature, capture probe concentration, label probe concentration, magnetic bead concentration, and salt concentration in the hybridization buffer. In addition, a nonradioactive label, digoxigenin (DIG) was tested for use in the RTC. KOG-500 RNA was labeled with DIG by in vitro transcription. The RTC procedure was performed exactly as for the ^{32}P-labeled probe. The Genius chemiluminescent system was used for detection of DIG probes.

The limit of detection of the RTC procedure was determined for both infectious virus and for purified viral RNA. The sensitivity of the two detection systems, DIG and ^{32}P, was also compared. To determine the limit of detection for infectious virus, serial ten-fold dilutions of dengue-2 virus, 20,000, 2000, 200 and 20 plaque forming units (PFU) were hybridized in the standard RTC procedure. The lowest amount of virus detectable by both types of probes was 200 PFU. To determine the limit of detection for purified viral RNA, dengue-2 viral RNA dilutions were subjected to RTC using ^{32}P or DIG-labeled probes. The lowest amount of RNA detectable with both probes was 10 ng. We have also used the RTC procedure for detecting virus in dengue-infected mosquitoes. Adult female Aedes aegypti mosquitoes were intrathoracically inoculated with 100 PFU of dengue-2 virus stock. After 7 days of incubation, mosquitoes were subjected to RTC. Hybridizations were performed with ^{32}P or DIG-labeled probes following the standard procedure. The procedure worked well; the signal/noise ratios of infected mosquitoes indicated that RTC was capable of detecting dengue-2 viral RNA in single mosquitoes with both ^{32}P and DIG-labeled probes (Figure 1a and b). Uninfected mosquitoes yielded no signal with ^{32}P or with DIG probes. These results indicate that use of this procedure in surveillance programs will allow detection of dengue virus in mosquitoes much more rapidly than current procedures. To determine if RTC was comparable to the virus isolation technique, a growth curve experiment was performed. C6/36 cells were inoculated with dengue-2 NGC virus at an MOI of 0.01. Samples of the cell culture media were taken at 0, 24, 48, 72 hours post infection. Samples were titrated by plaque assay to measure virus present in the medium; and assayed by RTC hybridization. RTC was slightly more sensitive, detecting virus at 24 hours while virus was not detected by plaque assay until 48 hours PI (Figure 2).

In summary, we have developed a novel hybridization procedure for the detection of dengue-2 viral RNA. This RTC procedure has the advantage of being highly sensitive. The specificity may be manipulated by choosing the desired probe sets. In addition, the RTC is performed on crude samples, eliminating the need for phenol-chloroform extraction of nucleic acids prior to hybridization. RTC is rapid, the entire procedure may be performed in one day. Future studies will include development of topotype-specific probes, use of the RTC to detect dengue viruses in human serum for diagnosis of dengue infections, and use of the RTC to detect dengue viruses in pools of mosquitoes for surveillance purposes.

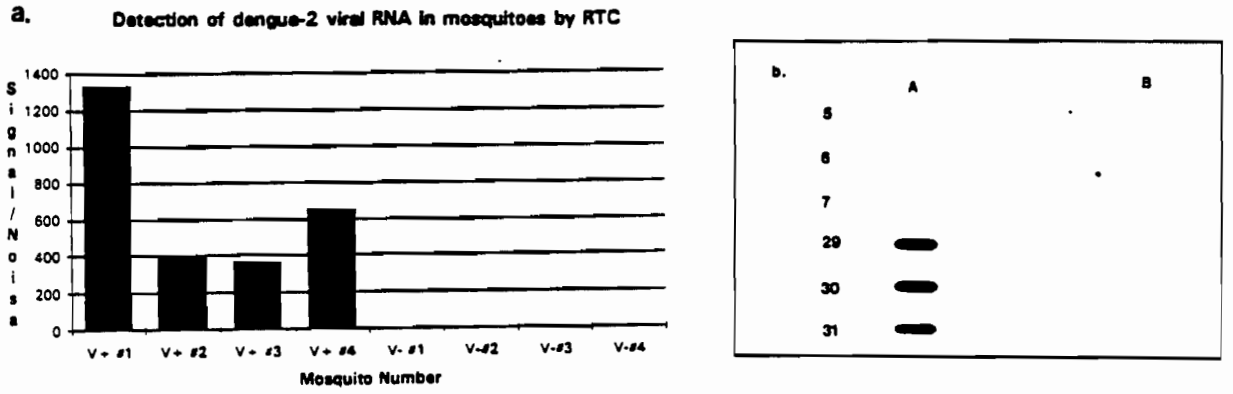

Figure 1. Use of the RTC procedure for the detection of DEN-2 viral RNA in mosquitoes. Mosquitoes were subjected to RTC with radiolabeled (a) or DIG-labeled (b) RNA detector probes. In (a), 4 each of virus infected mosquitoes (V+) or uninfected mosquitoes (V-) were subjected to RTC. In (b): #5,6,7 are uninfected mosquitoes; #29, 30, 31 are DEN-2 infected mosquitoes (average titer 4.8 $\log_{10}$ PFU per mosquito). Column A: complete hybridization complex reactions, column B: control reactions lacking capture probes.

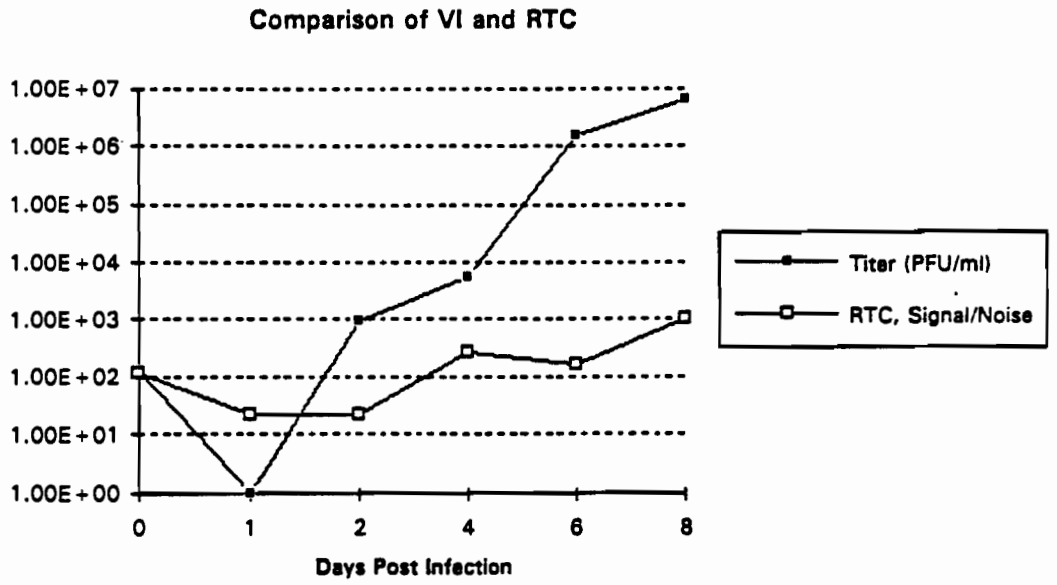

Figure 2. Comparison of the sensitivity of virus isolation and RTC. Samples of cell culture medium from dengue-2 virus infected cells were removed at 0, 1, 2, 4, 6 and 8 days post infection. They were subjected to plaque assay or RTC hybridization.

AN ENZYME IMMUNOASSAY FOR THE DETECTION OF EASTERN EQUINE ENCEPHALOMYELITIS (EEE) VIRUS

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Bioassays currently used to identify arthropod-borne viruses require special laboratory facilities and are time-consuming; at least one week is needed to isolate and identify most of these viruses.

Enzyme immunoassays (EIA) for detecting arboviral antigens show promise as rapid, sensitive, specific, and cost-effective alternatives to traditional bioassays.

A double antibody sandwich EIA was standardized for detecting EEE viral antigen, using EEE (C-110) and western equine encephalomyelitis (UPA) viruses. EEE mouse ascitic fluid was used both to capture and to detect viral antigen; the detecting antibody was peroxidase-labelled.

Samples previously detected from natural arbovirus foci, and stored at -70°C since then, were used in this study. Strains of EEE virus had been isolated from all of them and identified by neutralization tests.

To evaluate our system, all samples were inoculated into Vero cell cultures and these cultures tested at different intervals in order to determine infectivity titers and O.D. values. Positive samples had O.D. values >0.2. This EIA detects EEE virus at titers of 10^3 to 10^4 $TCID_{50}$ beginning 8-10 hours after inoculation. We did not observe cross-reactivity with western equine encephalomyelitis virus. The assay has good specificity and sensitivity.

Our evaluation is that this technique can be applied to the rapid and specific identification of EEE virus antigens.

S. Vázquez, J.L. Pelegrino, M.G. Guzmán, L. Morier, and A. Castillo

NUCLEOTIDE SEQUENCE AND CODING STRATEGY OF THE UUKUNIEMI VIRUS L RNA SEGMENT

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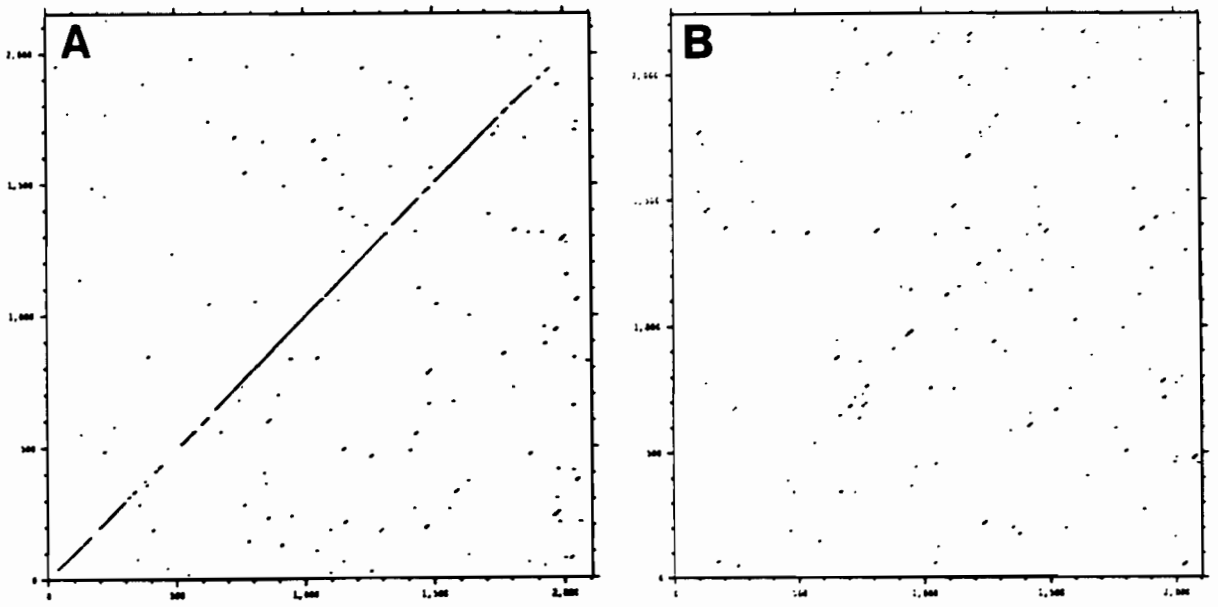
The complete nucleotide sequence of the L RNA segment of Uukuniemi virus has been determined from cloned cDNA. The L RNA is 6423 nucleotides in length, and is of negative polarity. The viral-complementary RNA contains a single large open reading frame of 2104 codons which corresponds to the L protein (M_r 241 039). Comparison with the L protein sequences of other members of the Bunyaviridae showed homology with the Rift Valley fever (RVF) phlebovirus (Fig.A: 38% amino acid identity), but no detectable similarity with bunyavirus (Fig.B), hantavirus or tospovirus L proteins. These data lend further support for the recent reclassification of uukuviruses and phleboviruses into the same genus, *Phlebovirus*, in the family Bunyaviridae. The L RNA sequence completes determination of the Uukuniemi virus genome: since the M RNA segment is 3229 and the S RNA segment 1720 nucleotides, the whole genome comprises 11 372 nucleotides. The Uukuniemi virus L RNA sequence has been deposited in the databases under the accession number D10759.



Dot matrix comparisons of the Uukuniemi virus L protein sequence (X-axis) with (A) Rift Valley fever virus L protein, and (B) Bunyamwera virus L protein.

Genatic variation among Japanese encephalitis viruses isolated in Thailand.

by
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Japanese encephalitis virus (JEV) has been traditionally classified using 5 JEV-specific monoclonal antibodies into at least 4 antigenic groups (Kobayashi *et al.*, 1984). However, this classification does not provide information about the epidemiological origin of a new genotype nor does it provide clear evidence of intratypic relatedness among strains of a single serotype.

Oligonucleotide fingerprinting of the virion RNA is another method in identifying the interrelationships among strains. This is based on genetics rather than antigenicity (Hori *et al.*, 1986; Banerjee & Ranadive, 1989). However, it is sensitive to small mutational changes in the genome. This limits its usefulness in demonstrating virus relationships to only within the same genotypic variants which are isolated not more than five years apart (Bishop, 1983; Trent *et al.*, 1983).

A more sensitive method of classifying viruses is the comparison of nucleotide sequences of a given gene or part of a gene. This is done by making pairwise comparisons of the sequences of the viruses studied. The sequence divergence is analysed by cluster analysis similar to that described by Fitch and Margoliash (1967). This method provides information on the genetic relationships, evolution and mutations of viruses that have been isolated over a long period of time. This method was successfully applied to influenza A virus (Bounagurio *et al.*, 1986) and poliovirus type 1 (Rico-Hesse *et al.*, 1987).

By sequence comparison, Chen *et al.* (1990) arbitrarily classified 45 isolates of JE viruses from different geographic areas in Asia into 3 genotypic groups. The first group comprised JE virus isolates from northern Thailand and Cambodia, the second comprised isolates from southern Thailand, Malaysia, and Indonesia, and the third from Japan, China, Taiwan, The Philippines, Sri Lanka, India and Nepal.

In our study, 25 strains of JEV isolated from various sources and locations in Thailand during the years 1979 to 1988 were obtained from Dr. Bruce Innis (AFRIMS, THAILAND) and their genetic relationships were investigated.

Total RNA was extracted from JEV-infected C6/36 cells by the method of Kautner and Lam (1992). Each RNA sample was subjected to direct RNA sequencing to determine 240 nucleotides in the preM gene (positions 456-695) by using the following two primers: 5'-GTGTCCTCACACATGTA-3' (map site 603-619) and 5'- TTGGAATGCCTGGTCCG-3' (map site 723-739).

Table 1 gives the details of 5 of 25 Thai JEV isolates and the control Nagayama strain. Figure 1 shows the sequences of the 240 nucleotides determined for the six isolates, and Table 2 summarizes the nucleotide differences among the isolates.

Table 1: Details of five Thai JEV isolates and the Nagayama isolate.

	ISOLATE	SOURCE	COLLECTED	PLACE	SEEDPASS
C1	NAGAYAMA	HUMAN BRAIN	1935	Japan	SMB-56
C2	KE83-100	HUMAN BRAIN	JULY 1983	northern Thailand	C6/36-3
C3	KP-0270	MOSQUITO	JUNE 1984	northern Thailand	C6/36-4
C4	B-2239/84	PIG	JUNE 1984	northern Thailand	C6/36-4
C5	B-2582/85	PIG	NOV 1985	northern Thailand	C6/36-3
C6	B-1065/83	PIG	JULY 1983	southern Thailand	C6/36-4

Table 2: Number of nucleotide changes among the JEV isolates

	C1	C2	C3	C4	C5	C6
C1		35	34	33	33	33
C2			3	4	4	28
C3				3	6	29
C4					4	30
C5						29

Figure 1: Sequences of 240 nucleotides within the preM region of JEV isolates listed in Table 1. Nucleotide differences from Nagayama strain are shown, dash indicates identical bases.

```
      456 461              481              501              521              541              561          575
C1      GUCAUAGCCUGCGCAGGAGCCAUGAAGUUGUCAAAUUUCCAGGGGAAGCUUUUGAUGACCGUCAACAACACGGACAUUGCAGACGUUAUCGUGAUUCCCACCUCAAAAGGAGAGAACAGA
C2      --A-C---U--U--C-----U------C-------C--U--A--A------C--------A----------------G-----A--------C-------------C--A------
C3      --A-C---U--U--------U------C-------C--U--A--A------C-------------------------G-----A--------C-------------C--A------
C4      --A-C---U--U--C-----U------C-------C-----A--A------C--------A----------------G-------------C-------------C--A------
C5      --A-C---U--U--C-----U------C-------C-----A--A------C--------A----------------G-------------C-------------C--A------
C6      -------UU-----U-----U------C---------------A--A---C---------------U--A-----C--G-----C--A--C--C-------------U--A------

      576 581              601              621              641              661              681          695
C1      UGUUGGGUCCGGGCAAUCGACGUCGGCUACAUGUGUGAGGACACUAUCACGUACGAAUGUCCUAAGCUCACCAUGGGCAAUGAUCCAGAGGACGUGGACUGUUGGUGUGACAACCAAGAA
C2      ----------A----------U--U----------A-----C----------------G-----AG-AG-------C--C-----A--------U--C-----C-----U------
C3      ----------A--G-------U--U----------A-----C----------------G-----AG-AG-------C--C-----A--------U--C-----C-----U------
C4      ----------A--G-------U--U----------A----------------------G-----AG-AG-------C--C-----A--------U--C-----C-----U------
C5      --C--------A----------U--U----------A-----C----------------G-----AG-AG-------C--C-----A--------U--C----------U------
C6      ----------A--------U--U--U----------A-----C----------------C---------CC---U-----C--------U-----U--C----------U------
```

Work is in progress to determines the 240 nucleotides in the preM region of each of the remaining 20 Thai JEV isolates. The sequences will then be compared by cluster analysis.

Acknowledgements

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REPORT FROM VIRUS LABORATORY, DEPARTMENT OF TROPICAL MEDICINE, MEDICAL RESEARCH INSTITUTE, KANAZAWA MEDICAL UNIVERSITY, UCHINADA, ISHIKAWA-KEN, JAPAN 920-02

PCR Products of DEN-1 VIRUS and Nucleotides Sequence of Viral Genome Coding for Structual Proteins.

DEN-1 virus (Mochizuki strain) cultivated in vero cells was used. Viral RNA was extracted from virions purified by sucrose density gradient centrifugation and phenol/chloroform-ethanol treatments. Three kinds of oligonucleotides were synthesized: P12 (5'-AACAGTTTCG-AATCGGAA-3'), P15(5'-CAACTTCCATGCTC-3') and P10(5'-GAG-TTCTCTGCCTTTCCAGT-3'). Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed for 40 cycles each using the primers. Technical specifics were essentially the same as those reported by other investigators.

Three main PCR products were detected: L (ca. 1kbp), M (ca. 0.65kbp) and S (ca. 0.35kbp). On agarose gel electrophoresis, the L fragment was most clear and was detected in the minimum amounts of materials equivalent to 0.001μl of infected culture fluid and corresponding to 0.1 PFU of virus. Similar results were obtained in experiments using crude culture fluids, instead of purified RNA. In the crude material cases, particularly in those using undiluted or lower diluted fluids, the data were not necessarily clear-cut, perhaps because of contamination of certain interfering factor(s) or RNAase-like elements. In an investigation conducted during the war time, it was noted that an intracutaneous injection of 0.1 ml of 10^{-5}-diluted dengue patient's febrile phase serum caused typical dengue symptoms in a healthy volunteer. Therefore, detection of DEN genome in patient's blood by the PCR methods is "theoretically" possible, although precautions should be taken to eliminate some reaction-inhibiting elements.

The PCR products were regarded to include DEN-1 virus structural proteins coding regions. The L fragment was selected for further test. The materials were extracted, treated with restriction endonucleases and then inserted into pUC19DNA. Nucleotides and amino acids sequences were determined. Data obtained of Mochizuki strain (isolated in 1943) were compared with 831-1 Philippine strain (isolated in 1983). The homology of these two strains were: 99% for C, 95% for preM and 97% for M. Apparently the structures of these regions have been well conserved in the two DEN-1 strains which were isolated 40 years apart.

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(Reported by S. HOTTA)

Molecular Characterisation of Mapuera Virus

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Mapuera virus was originally isolated from the salivary glands of a bat (*Sturnira lilium*) in Para, Brazil by Dra. Amelia Travassos da Rosa in 1979 and has been registered in the International Catalogue of Arboviruses since 1984. Within the catalogue, the virus was taxonomically unclassified but was noted as probably not being an arbovirus. Preliminary characterisation carried out by Zeller *et al.*, 1989 based on Electron Microscopic studies of virus infected cells and the cytopathic effects produced during growth in tissue culture suggested placement in the *Paramyxoviridae* family. To confirm the inclusion of Mapuera virus within this family and obtain information as regards its relatedness to other members of the family further characterisation has been carried out.

Mapuera virus was passaged in Vero cells and SDS-PAGE analysis of *in vivo* radioactively labelled viral polypeptides was performed.

In vivo labelling with ^{35}S-methionine and ^{14}C-Amino Acids revealed the presence of seven viral polypeptides (VP).

	Mol. wt.		Mol. wt.		Mol. wt.
VP1	66K	VP4	43K	VP7	200K
VP2	63K	VP5	35K		
VP3	49K	VP6	74K		

The viral polypeptides were further characterised by labelling with ^{35}S-cysteine, ^{32}P-orthophosphate and ^{3}H-glucosamine.

Labelling with ^{35}S-cysteine and ^{32}P-orthophosphate revealed VP5 to be cysteine rich and heavily phosphorylated. While labelling with ^{3}H glucosamine showed that VP2 and VP6 were glycosylated and revealed the presence of a further viral polypeptide of 36K which is also glycosylated and appears to comigrate with VP5.

The results of the various labelling experiments and comparisons with the protein profiles of other paramyxoviruses has allowed the preliminary identification of each of the viral proteins.

VP7 represents the L protein, VP6-HN protein, VP1-N protein, VP2-Fo protein, VP3-P protein, VP4-M protein and VP5 could be a non-structural protein related to the P protein. The other glycosylated protein of approximately 36K is proposed to be a proteolytic breakdown product derived from the HN glycoprotein.

The pattern of Mapuera viral proteins resolved by PAGE and the sizes of these viral proteins are almost identical to the protein profiles produced by paramyxoviruses such as mumps, SV5 and NDV and we suggest that it belongs to this subgroup of paramyxoviruses.

Electron microscopic studies of virus infected Vero cells confirmed the presence of nucleocapsid molecules with the classical herring bone morphology reported previously (Zeller *et al.*, 1989).

Mapuera viral RNA was also labelled with ^{32}P-orthophosphate in the presence of actinomycin D. The various viral RNA species were separated on a 1.5% vertical MOPS denaturing gel and visualised by blotting onto hybond N and exposing the membrane to x-ray film. A number of virus derived RNA species were observed. The pattern of bands produced was comparable to the RNA profiles of other recognised paramyxoviruses. The sizes of the various viral RNA transcripts were estimated to be HN-2000 nt, F-1900 nt, N-1650 nt, P/M-1200 nt and L-6500 nt. There were also several other faint bands observed on the film and these were considered to be bi- and tricistronic transcripts. No RNA species was observed which represented the SH gene found in the paramyxoviruses mumps and SV5.

In summary, the molecular characterisation of the proteins and RNA species induced by Mapuera virus, indicates that the virus belongs to the genus *paramyxovirus* in the *Paramyxoviridae*. Further experiments using monoclonal antibodies and comparisons of nucleotide sequences will further clarify its relationships to the other paramyxoviruses.

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ISOLATION OF A NEW LYSSAVIRUS FROM CATTLE IN NORTHERN AUSTRALIA

A growing number of members of the *Lyssavirus* genus have been isolated in Australia in recent years during arbovirus investigations. Adelaide River, Berrimah, Coastal Plains and Kimberley viruses have been recovered from cattle; Charleville, Humpty Doo, Oakvale, Parry Creek and Tibrogargan have been recovered from insects and bovine ephemeral fever virus has been isolated from cattle and insects.

A virus isolated from a bull near Darwin in 1985 has been characterised as a new lyssavirus. The virus was recovered after the bull's heparinised blood had been inoculated to embryonated chicken eggs with passage to the C6/36 line of *Aedes albopictus* cells and then to BHK-21 monolayers where CPE was observed. A second virus similarly recovered from another bull in 1986 appears identical.

The two viruses were compared with other members of the *Lyssavirus* genus at the Division of Vector - Borne Infectious Diseases, Fort Collins, Colorado by indirect immunofluorescence and plaque reduction tests. The viruses were shown to be related to, but distinct from, the rabies-related kotonkan virus. It is proposed that the new virus be named Koolpinyah, the local name for the electoral district from which the virus was recovered.

This is the second rabies-related rhabdovirus isolated in northern Australia. Adelaide River virus was recovered in 1981 from the blood of a steer pastured near Darwin and is related to rabies through Obodhiang and kotonkan viruses. No serological cross reactions have been observed between Koolpinyah and Adelaide River viruses.

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Identification of flaviviruses and alphaviruses from mosquitoes collected in New South Wales, Australia

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During surveillance for arboviruses in New South Wales, three flaviviruses and six alphaviruses were isolated from mosquitoes. By passage in BHK-21 and Vero cells, the isolates were amplified to sufficient titre to allow further studies, including biological characterization and identification.

The three flaviviruses were classified as flaviviruses by indirect immunofluorescence and identified to type by neutralization. As shown in Table 1, strain 96285 and 96346 are strains of Edge Hill virus, strain 96038 is a strain of Stratford virus. Isolate 96285 was from a pool of *Coquillettidia linealis* mosquitoes collected in 1982 south of Eden on the far south coast of New South Wales, and isolate 96346 was recovered from a pool of *Aedes vigilax* mosquitoes collected in 1983 from the Bateman's Bay area of the mid-south coast. Isolate 96038 was recovered from a pool of five species collected in 1981 in Conjola State Forest north of Bateman's Bay.

These are the first isolates of flaviviruses from the south coast of New South Wales. Although Edge Hill virus was recovered from *Aedes vigilax* on the mid-north coast of the state in 1969 and 1970 (Gard, Marshall, Woodroofe, Am J Trop Med Hyg, 22:551-560, 1973), Stratford virus has not been recovered previously from mosquitoes collected in coastal southeastern Australia. In this context, it is of considerable interest that in a seroepidemiological study of flavivirus infections in New South Wales, a high prevalence of antibody to Stratford virus was found in all areas of the state (Hawkes, Boughton, Naim, Wild, and Chapman, Med J Austral, 143:555-561, 1984.

The six alphaviruses were recovered from *Culex annulirostris* (strains 853 [February 20, 1990, Griffith], 1209 [March 5, 1990, Griffith], 1264 [March 6, 1989, Griffith], and 4890 [January 23, 1990, Wentworth]), *Culex annulirostris* and *Anopheles annulipes* (strain 1337 [February 20, 1989, Leeton]), and *Culex orbostiensis* and *Aedes notoscriptus* (strain 2209 [March 1, 1990, Maclean]) mosquitoes collected in New South Wales. These isolates were classified as alphaviruses by indirect immunofluorescence and attempts made to type them by neutralization. Final tests have not yet been done but strains 1209 and 4890, 1264 and 1337, and 853 and 2209 appear to represent three distinct virus types. None of the six isolates is identical to prototypes Sindbis, Getah, or Barmah Forest viruses but preliminary indications are that some may be closely related to Ross River virus and some may be closely related to Whataroa virus. Further efforts to identify these viruses continue to be made.

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Table 1. **Cross-reactivity of six prototype flaviviruses and three flavivirus isolates from Australian mosquitoes.**

Virus	Strain	Titer of antibody[a] to: (96346)	(96285)	EH	(96038)	STR	MVE	KUN	KOK	ALF
	96346	**160**	160	160	-[b]	-	-	-	-	-
	96285	160	**160**	80	40	-	20	20	-	-
Edge Hill	C-281	80	160	**80**	-	-	20	-	-	-
	96038	20	20	-	**320**	160	-	-	20	20
Stratford	C-338	20	-	-	320	**160**	-	20	20	-
Murray Valley enc.	11-A	-	-	-	-	-	**320**	40	-	40
Kunjin	MRM-16	-	-	-	-	-	80	**160**	20	-
Kokobera	MRM-32	-	-	-	-	-	40	20	**80**	20
Alfuy	MRM-3929	-	-	-	-	-	20	-	-	**≥640**

[a] Antibody preparations to MVE, KUN, KOK, STR, EH, and ALF viruses were hyperimmune mouse ascitic fluids; antibodies to strain 96346, 96038, and 96285 were hyperimmune mouse sera.

[b] - signifies <20

THE RESEARCH INSTITUTE FOR VETERINARY SCIENCE
ARBOVIRUS PROGRAM

The Research Institute for Veterinary Science (RIVS) has been studying arboviruses in Indonesia since 1985. The emphasis has been on bluetongue but other viruses have gradually been included in the program.

Indonesia comprises over 13,000 islands and a land area of 1.8 million sq km spaning a length of 5,600 km and a width of 1,600 km. The RIVS is located at Bogor, near Jakarta on Java. Some logistical problems where studies involve distant provinces are inevitable. Intensive studies are undertaken in the vicinity of RIVS while investigations at distant locations such as Irian Jaya are of a more extensive nature.

Initial arbovirus investigations by RIVS involved serum antibody surveys of ruminants. This early work laid a foundation for the present developing comprehensive program with components of serology, virology, entomology and pathology.

Viruses have been recovered from cattle and *Culicoides* spp. RIVS is still developing its arbovirus characterisation systems and isolates to date have been serologically identified with the generous assistance of several reference laboratories. Isolates include a number of bluetongue virus types and bovine ephemeral fever virus.

Vector light traps are sited near sentinel cattle. Most insects are collected into 70% alcohol but some near the laboratory are collected into PBS for virus isolation.

The main entomological emphasis has been on *Culicoides* spp. 35 species have been identified, several new to Indonesian records. Vector competence studies are planned to elucidate the important veterinary midges in Indonesia.

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Eastern Equine Encephalitis in Ohio During 1991

During August and September of 1991, an epizootic of eastern equine encephalitis in horses occurred in Wayne and Holmes Counties, Ohio. This is the first recorded epizootic of EEE in the State. The first horse case occurred on Sept.16, and the remaining cases occurred during the next 31 days. Twelve of the cases were confirmed through virus isolation, histopathology or serology. Seven cases were presumptive positives, based on symptoms compatible with EEE and proximity to the EEE focus. Seventeen horses died and two recovered. Most horses had no history of vaccination. However, in response to publicity regarding the epizootic, two horses received a single dose of EEE vaccine prior to the onset of clinical symptoms, one was vaccinated only two days prior to death.

The epizootic was centered around the Killbuck Wildlife Area. All of the horse cases occurred within an 8 km radius of the wildlife area, and 10 were within 3 km of the swamp basin. The 5306 acre wildlife area is in a shallow, U-shaped glacial outwash valley with a creek at the center. Approximately 56% of the wildlife area consists of marsh and swamp that is flooded during some portion of the year. The habitat surrounding the swamp and outside the wildlife area consists of mixed hardwood forests, old fields, pastures and cultivated lands. The region is primarily rural-agricultural and the human population is largely Mennonite and Amish. The nearest major town, Wooster, OH., is located approximately 5.5 km north of the marsh.

Mosquito collections were made using dry ice-baited CDC miniature light traps. Trapping was conducted in upland areas away from the swamp basin biweekly from May 29 through Sept. 24. Collections were made in the swamp basin on Sept. 10 and from Sept 20-24, after the horse cases were detected. The mosquito collections were pooled and tested for virus in vero cell culture.

Of 22,095 mosquitoes collected, representing 17 species, 43% were Coquillettidia perturbans. Aedes vexans and Cx. salinarius 22% and 17% of the total number collected, respectively. The remaining 14 species combined comprised 18% of the total collected, and none of these species comprised more than 5% of the total (Table 1). EEE virus was isolated from 1 pool of Cq. perturbans collected on Sept. 10 in the swamp basin; the MIR was 0.1/1000.

Birds were captured in Japanese mist nets placed at two sites in the swamp basin daily from Sept 20-24. Blood samples were tested for the presence of antibody to EEE with an IgM capture technique. Of 50 bird sera tested, three birds, representing three species, were positive for IgM to EEE virus (Table 2).

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Table 1. Mosquito collections from the 1991 EEE focus in Ohio tested for virus.

Species	No. Collected	No. Pools
Ae. species	31	4
Ae. cinereus	1	1
Ae. sticticus	1	1
Ae. triseriatus	7	6
Ae. trivittatus	445	37
Ae. vexans	4865	110
An. punctipennis	417	62
An. quadrimaculatus	1074	76
An. walkeri	1150	34
Cq. perturbans	9506	239
Cx. species	430	47
Cx. erraticus	51	15
Cx. pipiens	40	15
Cx. restuans	119	24
Cx. salinarius	3920	100
Cx. tarsalis	1	1
Cs. morsitans	1	1
Or. signifera	1	1
Ur. sapphirina	35	9
TOTAL	22,095	783

Table 2. Bird species tested for antibody to EEE using the IgM capture Elisa technique.

Species	No. Tested	No. Positive
Cardinal	5	0
Catbird	3	0
Black-capped chickadee	3	0
Carolina chickadee	2	0
White-breasted nuthatch	2	0
Ovenbird	1	0
Eastern wood pewee	4	1
Eastern phoebe	3	0
Song sparrow	6	1
Swamp sparrow	6	0
Thrush, unknown	1	0
Tufted titmouse	7	0
Downy woodpecker	6	1
Red-bellied woodpecker	1	0
TOTAL	50	3

INSITUT PASTEUR DE MADAGASCAR
ARBOVIRUS RESEARCH UNIT *

RIFT VALLEY FEVER ACTUALITY

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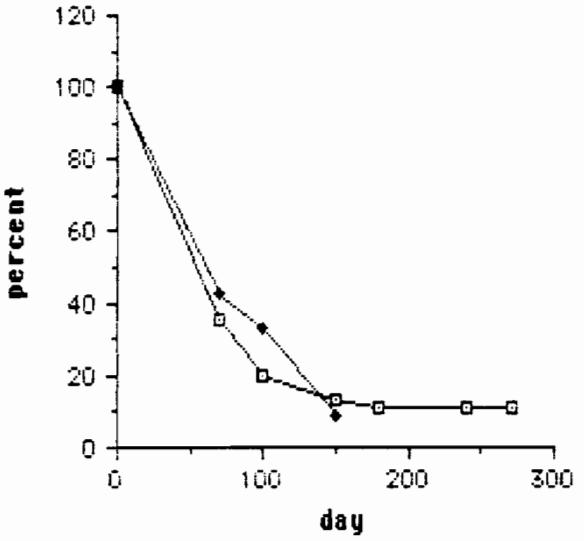
Duration of RVF virus IgM-class antibodies in cattle after natural infection.

During the RVF epizootic which occured in central highlands of Madagascar in 1991, a serological survey was conducted in bovines to precise the duration of specific IgM-class antibodies after natural infection.
195 bovines (37 recently aborting females and 158 other bovines) with positive sera for IgM at day-0 (D0)were sampled at D70, D100, D150, D180 and D240. Sera were tested for IgM-specific RVF virus antibody by an ELISA-IgM capture assay.
Cumulative probability of persistance of IgM-class antibodies is presented in Figure 1. IgM decreased progressively, and IgM became undetectable in 90 percent of cases by day-150.

Figure 1. Cumulative probability of persistance of RVF virus IgM-class antibodies in cattle after natural infection.



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Antigenic analysis of Rift Valley fever virus strains isolated in 1991 in Madagascar

In 1991, RVF virus strains isolated when an epizootic occured in central highlands of Madagascar were analysed for antigenic variations using monoclonal antibodies (kindly provided by J.F. Smith; USAMRIID).

Virus strains: 19 RVF virus strains (17 bovine isolates and 2 human isolates), and 3 prototype strains (MgH 824 from Madagascar, ArB 1976 from Bangui, and ZH 501 from Egypt) were used at mouse passage 3 and tissue culture passage 3.

Monoclonal antibodies(MAbs): RVF virus strains were tested by immunofluorescence assay using a panel of 8 MAbs directed against the glycoprotein G1 (3B4) and G2 (4D4), the nucleocapsid NC (R1P2E7 and 9G3), the non-structural protein NSP31 (3C3).

Results: the results of indirect immunofluorecence assay are presented in Table I.

In This study the data revealed that the 19 isolates appear to be antigenically identical Difference between these strains and RVF virus strains isolated in Madagascar in 1979 were found in the reactivity of R1P2E7 MAbs specific of egyptian and previous madagascan strains.
The results suggest the existence of different origins of RVF virus in Madagascar.

Table I Antigenic analysis of RVF virus strains isolated in Madagascar using monoclonal antibodies (USAMRIID).

souches	anti R1P2	NC 9G3	anti NSP 3C3	anti G1 3B4	anti G2 4B4	9B6	3B9	1F6
E501	+	+	+	+	+	-	+	+
ArB 1976	-	+	+	+	+	-	+	+
MgH 824	+	+	+	+	+	-	+	+
MgAn990	-	+	+	+	+	-	+	+
MgAn 991	-	+	+	+	+	-	+	+
MgAn 992	-	+	+	+	+	-	+	+
MgAn 993	-	+	+	+	+	-	+	+
MgAn 994	-	+	+	+	+	-	+	+
MgAn 995	-	+	+	+	+	-	+	+
MgAn 996	-	+	+	+	+	-	+	+
MgAn 997	-	+	+	+	+	-	+	+
Mg An 998	-	+	+	+	+	-	+	+
MgAn 999	-	+	+	+	+	-	+	+
MgAn 1000	-	+	+	+	+	-	+	+
MgAn 1001	-	+	+	+	+	-	+	+
MgAn 1002	-	+	+	+	+	-	+	+
Mg H 1003	-	+	+	+	+	-	+	+
MgAn 1004	-	+	+	+	+	-	+	+
MgAn 1005	-	+	+	+	+	-	+	+
MgAn 1006	-	+	+	+	+	-	+	+
MgAn 1007	-	+	+	+	+	-	+	+

(-) < 1/16 by IFA.

ISOLATION OF EASTERN EQUINE ENCEPHALITIS VIRUS FROM AEDES ALBOPICTUS FROM POLK COUNTY, FLORIDA.

During June 6-10, 1991, as part of an ongoing study to identify the sources of Aedes albopictus bloodmeals, Mark L. Niebylski and John-Paul Mutebi from the University of Notre Dame collected mosquitoes in and around a tire dump in Polk County, Florida. The collections were made with a Nasci aspirator and yielded 9393 Aedes albopictus mosquitoes that were sent to the Medical Entomology-Ecology Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control, Fort Collins, in December 1991. A total of 9350 mosquitoes were tested, in 96 pools, for virus isolation by plaque assay in Vero cell culture. Forty-three blood-fed specimens were tested separately for bloodmeal identification. The specimens tested for virus yielded 14 virus strains identified as eastern equine encephalitis (EEE) virus by indirect fluorescent antibody test using a panel of alphavirus monoclonal antibodies including EEE virus complex-specific (1B1C-4) and North American EEE virus-specific (1B5C-3) monoclonal antibodies. The virus strains were reisolated from the original mosquito pools by intracranial inoculation into 1- to 3-day-old suckling mice. Two representative isolates were confirmed as EEE virus by plaque-reduction neutralization test. Results of the bloodmeal identifications were 31% bovine, 24% unidentified mammal, 19% deer, 14% human, 7% raccoon, 5% rabbit and 2% passeriform birds.

This is the first documented isolation of EEE virus, or any other virus of public health or veterinary importance, from Aedes albopictus collected in the United States. In the United States, EEE is the rarest of the mosquito-borne arboviral encephalitides but has a human case-fatality rate of approximately 30%. The virus is maintained in fresh-water-swamp habitats in an enzootic cycle involving mosquitoes, principally Culiseta melanura, and a variety of bird species. During 1991, heavy spring rains in northern Florida led to exceptionally large populations of Cs. melanura as well as mosquito species that serve as epizootic vectors. Consequently, Florida experienced early, widespread EEE virus activity with 70 equine cases reported by the beginning of July, the highest reported in a season by that time. Polk County reported four confirmed EEE cases in equines, three of them with onset in May and June; date of onset for the other case is unknown. Therefore, epizootic transmission of EEE virus was occurring in Polk County during the same period that infected Aedes albopictus mosquitoes were collected at the tire dump. In addition, the Florida Department of Health and Rehabilitative Services (HRS) confirmed five human cases of EEE among elderly residents during this period, but all were from an area approximately 125 miles north of Polk County.

Information on the distribution, vector competence, and feeding habits of Aedes albopictus, along with findings reported here regarding virus isolations from field-collected specimens, suggest that Aedes albopictus may become an epizootic and epidemic

vector of EEE virus. Plans are underway by the Florida Department of Agriculture, the Florida Medical Entomology Laboratory, the Florida HRS, the Polk County Environmental Services, the University of Notre Dame, and CDC to improve surveillance for EEE in Polk County and to conduct collaborative studies during the summer of 1992 to more clearly define the role of Aedes albopictus in the EEE transmission cycle.

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Rift Valley fever Crimean-Congo hemorrhagic fever virus activity in Senegal

1-RVF:

RVF IgG antibody prevalence in domestic ungulates in Senegal decreased since the 1987 Mauritanian outbreak. In the Senegal river basin, the prevalence in small ruminants was 23.9% in 1989 and 6.2% in 1991. Such decrease could be explained by a low level of virus circulation and the rapid turnover of the animals. However, the prevalence of RVF IgG antibodies in sheep and goats less than two years old proves some virus transmission in an enzootic cycle without any disease. *Aedes* (*Neomelanoconion*) *mcintoshi* was present in the region just after the flooding of the seasonal pools. It will focus attention as potential vector of RVF virus as described in East Africa. The observation of few RVF seroconversions in tagged animals during the dry season (February - March) when the zoophilic mosquitoes were less abundant, could indicate a non-mosquito transmission. The role of sandflies as vectors, the roles of bats and rodents as reservoirs, will require further studies.

2-CCHF:

In 1991, two locations for CCHF virus active transmission were recorded. In northern Ferelo, Tatki (16 14' N, 15 17' W) in the sahelian area, CCHF prevalence rate was 81.2% (69/85) and in small ruminants 17.1% (12/70). Minimal infection rate in *Hyalomma marginatum rufipes* adult ticks was 0.98%, 10 isolates of CCHF virus from 1025 ticks sampled in 63 pools. In Bandia (15 35' N, 17 W), in sudanian area, two isolates of CCHF virus were recovered from *Hyalomma marginatum rufipes* adult ticks tested in 168 pools; 0.11% of *Amblyomma variegatum* adult ticks contained CCHF virus; 0.12% of *Rhipicephalus guilhoni* adult ticks contained CCHF virus; and 0.7% of *Rhipicephalus evertsi evertsi* adult ticks yielded 4 isolates of CCHF virus.

CCHF antibody seroconversions were recorded in 4 of 12 goats monitored monthly in Bandia in November and December, 1991. IgM was present for less than 2 months.

Otherwise, from the soft tick *Alectorobius sonrai* collected in 1987 in the Bandia area, CCHF virus was isolated and recovered associated with other nairoviruses: Bandia, commonly isolated from rodents and soft ticks in the area, and Dugbe. The mixed virus isolations of Dugbe-Bandia was also obtained. CCHF transstadial transmission in nature was observed with *Hyalomma* nymphs collected in an *Arvicanthis* sp. rodent burrow on 12/31/87 in Bandia. The nymphs were allowed to molt and then tested for virus isolation with a positive result.

Report from Arbovirus Laboratory, Pasteur Institute of Dakar, B. P. 220, Dakar, Senegal (H. Zeller, J. P. Digoutte) ORSTOM Medical Zoology Laboratory, ORSTOM, B. P. 220, Dakar Senegal (J. L. Camicas, J. P. Cornet, D. Fontenille, M. Traoré-Lamizana)

READERS PLEASE NOTE: QUITE BY ACCIDENT, THE EDITOR SEVERELY DAMAGED THE ORIGINAL TYPESCRIPT OF THIS REPORT. HE HAS MADE EVERY EFFORT TO RECONSTRUCT THE ORIGINAL REPORT; HOWEVER, ALTHOUGH THE REPORT SHOWN ABOVE IS ACCURATE, CERTAIN WORDS WERE OMITTED AND THE REPORT IS NOT ABSOLUTELY COMPLETE. HE APOLOGIZES TO THE AUTHORS AND TO THE READERS.

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

Arbovirus Surveillance in New Jersey, 1991

During the 1991 surveillance period from June into October, 614 mosquito pools containing up to 100 mosquitoes each were tested for viruses in day old chicks. There were 15 mosquito pools positive for Highlands J (HJ) virus and Eastern encephalitis (EE) was isolated from 21.

HJ mosquito activity is summarized in Table 1. The late July collections gave the first isolates with continued observation of HJ activity into early October. There were 15 isolates from Culiseta melanura at 5 sites.

Table 2 summarizes the collection area totals, species of mosquito and time of collection for the EE isolates. Activity began with mid-August collections and continued into October. All of the 21 isolates were from pools containing Culiseta melanura mosquitoes at 7 sites.

EE isolates were also made in September and October in 2 horses in southern coastal counties.

(Shahiedy I. Shahied, Bernard F. Taylor, Wayne Pizzuti)

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Table 1
1991
HJ MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	7/19	7/26	8/2	8/9	8/16	8/23	8/30	9/7	9/14	9/21	9/28	10/5	AREA TOTALS
Bass River	Cs. melanura	1	3	2	2		1	1						10
Dennisville	Cs. melanura		1											1
Forked River	Cs. melanura						1							1
Hammonton	Cs. melanura						1	1						2
Waterville	Cs. melanura												1	1
WEEKLY TOTALS		1	4	2	2	0	3	2	0	0	0	0	1	15

Table 2
1991
EE MOSQUITO POOL ISOLATES
FOR WEEK ENDING

AREA COLLECTED	MOSQUITO SPECIES	8/16	8/23	8/30	9/7	9/14	9/21	9/28	10/5	AREA TOTALS
Bass River	Cs. melanura		1	1	4					6
Centerton	Cs. melanura				1		1			2
Dennisville	Cs. melanura	1	1				1		1	4
Forked River	Cs. melanura			1						1
Hammonton	Cs. melanura	1								1
Marlton	Cs. melanura						1	1		2
Waterville	Cs. melanura			1			1	2	1	5
WEEKLY TOTALS		2	2	3	5	0	4	3	2	21

INCREASED ARBOVIRUS ACTIVITY IN THE NORTH OF WESTERN AUSTRALIA DURING THE 1990/91 WET SEASON.

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Several medically important arboviruses are known to circulate in mosquito-vertebrate cycles in the north of Western Australia. Murray Valley encephalitis virus (MVE) is recognised as the major aetiological agent of Australian encephalitis (AE), although a few cases may be caused by the closely related Kunjin (KUN) virus. MVE, a flavivirus of the Japanese encephalitis - West Nile virus subgroup, causes occasional, severe epidemics of AE in south-east Australia, and sporadic cases of AE in northern Australia and Papua New Guinea (Marshall, 1989). Both viruses are considered to be endemic in parts of the Kimberley and the Northern Territory but are epidemic in the Pilbara and Gascoyne regions of Western Australia and probably in northern Queensland.

Ross River virus (RRV) and Sindbis (SIN) virus are two alphaviruses that have been isolated from mosquitoes in Western Australia. Both viruses are widely distributed throughout Australia. RRV is recognised as the causative agent of epidemic polyarthritis and has been reponsible for several major epidemics throughout Australia (Kay and Aaskov, 1989). Human infections caused by SIN virus appear to be rare, but isolated clinical cases have been reported from Victoria and Queensland (Doherty *et al.* 1969; Guard *et al.*, 1982).

The 1990/91 monsoonal wet season has been exceptional in northern Australia, with near record rainfalls in the north of the Northern Territory and heavy falls in the north of Western Australia, particularly in the south-east and west Kimberley. Increased rainfall has caused large scale flooding in some areas with an associated increase in mosquito numbers and a subsequent increase in arbovirus activity. Arbovirus activity in the remote north of Western Australia is monitored on a year round basis using sentinel chicken flocks located in various towns in the Kimberley and Pilbara (see Fig. 1) and by yearly mosquito collecting trips to the region. A large number of mosquitoes were caught using EVS-CO_2 traps in the Kimberley region of W.A. in April 1991. The majority of mosquitoes trapped were *Cx. annulirostris* and to date only 15% of the total catch has been identified. There have been 12 isolates of MVE, 4 isolates of KUN and 79 isolates of SIN confirmed from Broome, Kununurra and Balgo.

An increased flavivirus seroconversion rate in the sentinel chicken flocks in the Kimberley in 1991 gave an early warning of an increase in MVE and KUN activity throughout the Kimberley. This activity later spread to areas of the Pilbara. In addition a large number of sentinel cattle from Kununurra and Broome were found to have seroconverted to MVE over 1990/91 wet season.

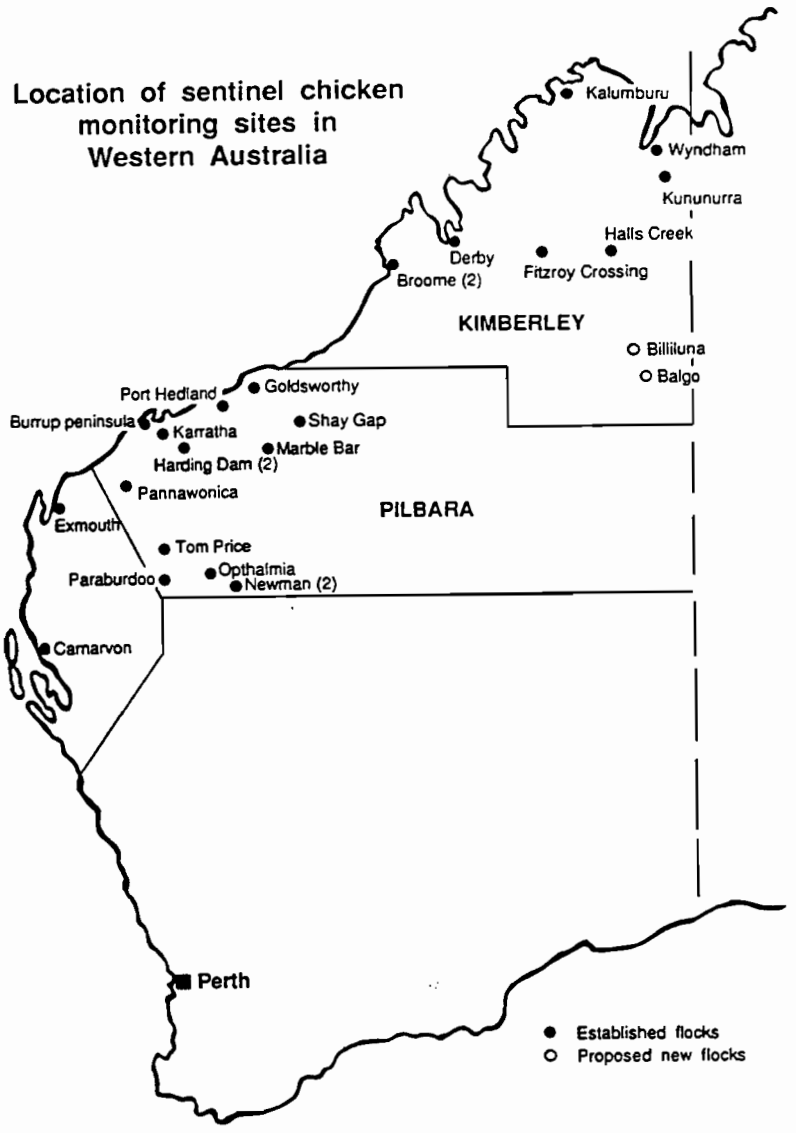
There has also been a significant increase in RRV activity throughout the Kimberley over the 1990/91 wet season which has resulted in the most recorded cases of epidemic polyarthritis (55) from the region. This compares with an annual average of 15 cases in previous years. A large outbreak of epidemic polyarthritis (some 200 cases), was also reported from the Northern Territory (Whelan *et al.* 1991).

The last major epidemic of AE in south-east Australia was in 1974 and since then there has been no MVE activity in the area although there has been evidence of KUN virus activity in some interepidemic years. Since 1974 the majority of cases of AE (24/35) have occurred in Western Australia with three recent fatalities. Most cases, particularly severe or fatal ones, have occurred in young Aboriginal children (Mackenzie *et al.* submitted for publication). Five cases of AE, two from

Western Australia, one from Queensland and two from the Northern Territory have been reported in 1991 (Mackenzie *et al.* 1991; Aaskov and Phillips, personal communication). Four of the cases were caused by MVE virus with one fatality recorded from Balgo (approximately 200 km south of Halls Creek in Western Australia). One mild case of AE reported from Kununurra in Western Australia was caused by KUN virus.

Additional evidence of an increase in flavivirus activity was obtained from an on-going serological study of the Aboriginal community at Billiluna in Western Australia. Billiluna is situated in the south-east Kimberley, approximately 150 km south of Halls Creek and is separated from the rest of the Kimberley by desert terrain. Seven children from this community have recently seroconverted to MVE and five others showed evidence of a recent infection with a flavivirus other than MVE. This increase in flavivirus activity recorded after heavy rain and flooding in the area is the first evidence of MVE virus activity in Billiluna since 1981 when several cases of AE were reported. This evidence suggests that under certain environmental conditions, particularly heavy rainfall, the virus can be re-introduced into epidemic areas, probably by viraemic waterbird movement from virus endemic areas of the Kimberley.

FIGURE 1.



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Report from Department of Arbovirus,Institute of Virology,Chinese Academy of Preventive Medicine, Beijing,100052.

SINDBIS VIRUS ISOLATED IN CHINA FOR THE FIRST TIME

1Liang Guodong, 2Li Qiping, 1He Ying, Chen Boquan, 2Xie Xinchu, 1Zhao Zijiang, Chen Li, Huang Yijun, 2Zhi Qi, Ma Li, Ba-Te and A-Bulikemu.

A strain of alphavirus,XJ-160,was isolated from a pool of Anopheles species collected from Yi Li area,Xin Jiang Provence,P.R.China. The identification of the virus was indicated by the following data:

1. Illness and death in suckling mice;evident cytopathic effect in C6/36 and BHK cells;
2. Sensitivity to acid and ether but resistant to 5-FduR; agglutination of sheep red blood cells;and
3. Spherical enveloped particles characteristic of alphavirus approximately 58.7 nm in diameter.

The virus was antigenically related to the alphaviruses Sindbis(SIN),Chikungunya(CHIK),and Eastern Equine Encephalomyelitis(EEE),particularly to SIN.The neutralising index of SIN antibody with XJ-160 virus,as measured in suckling mice,was 100,000,while those of other viruses were lower by a factory of 10.

Sindbis virus is isolated in mainland China for the first time.

EASTERN EQUINE ENCEPHALOMYELITIS VIRUS ISOLATED IN CHINA

2Li Qiping, 1Liang Guodong, 2Xie Xingchu, Zhi Qi, 1Chen Boquan, 2Ma Li, Ba-Te, Ye-Er-Keng, A-Bulikemu, 1He Ying, Zhao Zijiang, Chen Li and Huang Yijun.

In 1991, a strain of alphavirus,XJ-91031,was isolated from a pool of Ixodes persulcutus collected from the Bo-Er-Ta-La area of Xinjiang Provence,P.R.China.The virus identification was determined by the following data:

1. Illness and death in suckling mice and 3-6 week old mice; evident cytopathic effect in BHK,Vero and C6/36 cells; sensitivity to acid and ether,resistant to 5-IduR;and spherical enveloped particles characteristic of alphavirus approximately 70 nm in diameter.
2. The virus was found to be antigenically related to the alphaviruses,Eastern Equine Encephalomyelitis(EEE), Sindbis(SIN),Chikungunya(CHIK),Ross River(RR),Mayaro (MAY) and Getah(GET).The neutralising index of EEE antibody with the XJ-91031 virus,as measured in 3 week old mice,was 1,000, while that of SIN was 100 and those of CHIK,RR,MAY and GET were yet lower by a factory of 10.

This is the first reported isolated of EEE virus in mainland China.

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THE VECTORS OF ARBOVIRUSES DISCOVERED IN CHINA

Liang Guodong Chen Boquan

The vectors of arboviruses discovered in China are the following:

Virus	Vector
Japanese Enc.	
	Culex tritaeniorhynchus
	C. bitaeniorhynchus
	C. pseudovishnui
	C. whitmorei
	C. theileri
	C. annulus
	C. gelidus
	Aedes albopictus
	Ae. chemulpoensis
	Ae. vexans
	Anopheles sinensis
	Mansonioides uniformis
	Lasiohelea taiwana
Dengue virus	
	Ae. aegypti
	Ae. albopictus
	C. quinquefasciatus
RSSE virus	
	Ixodes persulcatus
	Haemaphysalis concinna
	Dermacenter silvarum
	De. marginatus
CHF-Congo virus	
	Hyaloma asiaticum
Orbivirus(12-segment RNA)	
	C. tritaeniorhynchus
	C. fuscocephala
	An. annularis
	An. sinensis
	An. vagus
	An. maculatus
	Ae. albopictus
	Ae. annandalei
	Ae. desmotes
	Ae. gardnerii imitator
	Ae. veaans
	Mansonioides uniformis
	Heizmannia reidi
	Ixodes persulcatus
	De. silvarum
	De. niveus
	Hyaloma asiaticum

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2. Institute of Endemic Diseases and Control in Xinjiang, Xinjiang Provence, 830002, P.R.China.

REPORT FROM THE NEW YORK STATE DEPARTMENT OF HEALTH
WADSWORTH CENTER FOR LABORATORIES AND RESEARCH

ARBOVIRUS SURVEILLANCE IN NEW YORK STATE: 1991

Alphavirus activity: Following a record outbreak of Eastern Equine Encephalitis (EEE) in 1990, in which 126 virus isolates were obtained from infected horses, sentinel pheasants, wild birds and mosquitoes in both upstate and downstate areas of New York, EEE virus activity was resumed the following year in the upstate Syracuse region.

Forty-two isolations of EEE virus were obtained from 1,653 pools of 97,019 mosquitoes and 139 non-human vertebrate specimens collected between 6/03/91 and 9/26/91 in upstate New York. Thirty (75%) of the 40 isolates from mosquitoes were obtained from the enzootic vector, Culiseta melanura, which is primarily responsible for the transmission of EEE virus among wild passerine birds. The remainder came from Culiseta morsitans (1), Aedes canadensis (1) and Coquilletidia perturbans (8); the two latter species, which have a broad host range including mammals, are considered to be epizootic vectors of EEE virus in this area. Infected mosquitoes were collected from all 4 counties; i.e., Madison, Oneida, Onondaga and Oswego, in the central New York surveillance region. During 1991, populations of Cs. melanura and Coq. perturbans, the principal enzootic and epizootic vectors, respectively, were higher than normal and emerged 2 to 3 weeks sooner than usual, resulting in the earliest EEE virus isolation from mosquitoes recorded in New York State.

Of 11 suspect equine cases investigated in 1991, EEE virus infection was confirmed in 2 horses from Oswego County by isolation of the virus from post-mortem brain tissue. One horse, which had been vaccinated for EEE and WEE in September, 1990, died on 7/23/91 within 24 hours of onset of illness. The other horse, which had no history of vaccination, was from Hastings in the Village of Central Square where a resident succumbed to fatal infection in 1971. A presumptive diagnosis of EEE was made in 5 other cases on the basis

of clinical symptoms, vaccination history and serologic findings; namely, a hemagglutination-inhibition antibody titer of 1:320 or greater to EEE virus. It is noteworthy that the first equine case of EEE encountered in the outbreak of 1991 occurred in mid-July, one month earlier than the first case detected in 1990. In contrast to the previous year, wild birds were not routinely sampled in 1991 and there was no evidence of EEE virus infection in sentinel pheasants from the area.

Bunyavirus activity: Two of the mosquito pools from upstate New York tested for EEE virus yielded Bunyavirus isolates. Jamestown Canyon (JC) virus was obtained from a pool of 100 Ae. canadensis collected 7/25/91 in Oswego County and Cache Valley (CV) virus was isolated from 44 Coq. perturbans collected 8/6-8/91 in Onondaga County. In addition, a total of 626 pools of 40,841 mosquitoes from 3 other counties in western (Erie) and southeastern (Dutchess, Suffolk) New York were tested for Bunyaviruses; isolations were obtained from 5 pools of 412 mosquitoes collected between 6/27 and 8/16/91 in Suffolk County from the following species: Aedes cantator (2 JC) and Coq. perturbans (1 JC, 2 CV).

Margaret A. Grayson, Ph.D. and Leo J. Grady, Ph.D.

REPORT FROM THE VECTOR-BORNE AND SPECIAL PATHOGENS UNIT

LABORATORY SERVICES BRANCH
ONTARIO MINISTRY OF HEALTH
TORONTO, ONTARIO, CANADA

Events:

1. Reorganization of the Virus Laboratory included changes in the structure of this unit. The laboratories of Arboviruses and Special Pathogens and of Electron Microscopy were grouped into one structure, the Vector-Borne and Special Pathogens Unit which presently includes:

   - The Laboratory of Arthropod-Borne Diseases: engaged in the diagnosis, identification and investigation of arthropod-borne viral and rickettsial infections.

   - The Maximum Containment Laboratory (MCL): engaged in investigating high and maximum hazard agents (Biohazard Level 3,4) suspected and diagnosed cases of such diseases.

   - The Laboratory of Electron Microscopy: engaged in the direct detection and morphological identification of viruses. This laboratory also undertakes studies of relevant outbreaks and provides such services to other sections of the Virology Laboratory.

   - These three components of the Unit also undertake investigations in their respective and integrated scope, as well as training visiting microbiologists.

   - The Laboratory and Office of Arboviruses and Special Pathogens were relocated adjacent to the MCL, and the new site was renovated to serve as a monitoring and support laboratory for the same.

2. The Maximum Containment Laboratory was further upgraded with installing full capabilities for suit mode operation. Breathing air is supplied through a duplex compressor system with two units which operate alternately and which also back-up each other. If the compressed air supply is impaired a line of twelve cylinders of emergency breathing is activated. This line consists of two sets of six cylinders each; each set is controlled through a manifold system.

   The MCL will usually operate in a cabinet mode and the suit mode capability is available in cases of spills, emergencies or unusual situations.

- Surveillance:

During a one year surveillance program ending in 1/4/92 we investigated 2,121 samples from 1,510 suspected cases of vector-borne disease.

This period marks the highest frequency of patients with travel history, 419/1510 (27%), since this program was established.

Samples were examined for evidence of vector-borne viral infections by one or more of the following techniques: hemagglutination inhibition, complement fixation, microneutralization and immunofluorescence assay (HAI, CF, MNT, IFA). Such tests involved 6-8 antigens or viruses, depending on the suspect diagnosis and circumstances, these are:

Flaviviruses:
St. Louis encephalitis, Powassan, dengue, yellow fever and Banzi.

Alphaviruses:
Eastern, Western and Venezuelan equine encephalitis.

California Group Viruses:
Snowshoe hare (SSH) and Jamestown Canyon (JC).

Viral Hemorrhagic Fevers (VHF):
Congo-Crimean, Rift Valley, Ebola Zaire, Ebola Sudan, Lassa, Marburg and Hantaan (hemorrhagic fever with renal syndrome).

Tests for rickettsial infections were done by IFA and included: (a) Phase 1 and Phase 2 of Q Fever for the three classes of immunoglobulins, IgG, IgM, IgA; the last class is of particular significance in cases of endocarditis; (b) Rocky Mountain spotted fever (RMSF) which cross-reacts with R. conorii, the causative agent of Mediterranean spotted and South African tick bite fevers, murine typhus. Tests were done for IgG and IgM classes only.

There were 189 seropositive patients (12.5%) to one or more of the above killed antigens or live viruses. Thirty nine of the 189 seropositive patients (20.6%) had diagnostic findings. The rest had antibodies not necessarily related to the then current or recent diseases. Twenty two of these 39 cases (56%) were diagnostic of arboviral infections and 16 (41%) of rickettsial diseases. Serum referred by the Missionary Health Institute, from a patient who died in Jos, Nigeria was IgM positive to Lassa and confirmed diagnosis of this disease. These data are given in Table 1.

The seropositive patients to above antigens or viruses included 82/167 (49%) to flaviviruses, 78/167 (47%) to the California Group, 8 (5%) to Alphavirus(es).

This is the highest number of seroreactive findings to alphavirus(es) in one year, the eight patients had histories of travel. The specificity of this reactivity is being ascertained. Seroreactivity, otherwise, to alphaviruses is usually rare in our surveillance data.

Arthropod-Borne Viral Infections:

Seventeen patients had diagnostic findings of flavivirus disease, dengue in the majority of cases. The serological response was usually associated with history of travel to flavi or dengue active area and clinical diagnosis of this disease.

Seventy eight patients were seropositive to the California Group Virus. Twenty of them had histories of travel, association with seropositive reaction in 18 cases was not ascertained. There were five cases with presumptive diagnosis of California Group virus, 2 snowshoe hare and three untyped. Antibody presence in the remaining 73 cases, can be categorized as likely not related to the patients' disease. It should be noted that because of the lack of HAI ether-tween extracted antigen for the CGV, the microneutralization test, which is both sensitive and specific, has been used both as a screening and typing test. The 78 seropositive cases were reactive in 39 cases by HAI, in 34 by MNT and in 5 cases by both tests. Data on 39 of these showed 9 Jamestown Canyon, 9 snowshoe hare, 16 type indeterminate and 5 in typing or inconclusive.

Rickettsial Infections: The number of samples received and cases investigated for rickettsial infections increased more than 10 and 15 folds those of 1986, the year the current testing methodolodgy was introduced. This year, 354 cases were investigated for rickettsial infections, 21 (5.5%) were seropositive with 16/21 (761%) having diagnostic significance. Ten were for Q fever and 6 for Rocky Mountain and Mediterranean spotted fevers, South African tick bit fever and murine typhus. The latter group had travel history to disease endemic areas and included two from Portugal. Rickettsia like bodies were identified in the squash of a tick removed from a traveller from Australia who likely acquired Queensland tick fever. Reagents for the diagnosis for Erlichiosis were acquired and the test can be performed now in suspected cases.

Histories of Travel and Suspect Cases of Exotic Diseases: During this period, 419 patients had histories of travel, the highest percentage we have had. Thirteen had diagnostic findings consistent with flavivirus infections, two with CGV and six with rickettsial disease. Laboratory diagnosis of Lassa fever was established in a case where the patient died in the Missionary Hospital in Jos, Nigeria. Hemorrhagic fevers (Lassa, Hantavirus) were ruled out in three other requests.

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13/5/92

TABLE 1
SEROLOGICAL FINDINGS IN CASES WITH SUSPECT VECTOR-BORNE DISEASE

SEROPOSITIVE FINDINGS ON		CASES INVESTIGATED		
		Diagnostic		
AGENT	TOTAL SEROPOSITIVE	CONFIRMED	PRESUMPTIVE	LIKELY NOT RELATED TO DISEASE
Alphavirus *	8 ** (7 + 1)	0	0	8 ** (7 + 1)
Flavivirus	82	5	12	65
California Group Virus	78	0	5	73
Q Fever	15	4	6	5
Rocky Mountain/ Murine typhus African Tick-bite Mediterranean/ Fever	6	3	3	0
Viral Hemorrhagic Fever	1	1 Lassa	0	0
TOTAL	189	13	26	150

* Specificity to be ascertained.
** Includes one seropositive to California Group Viruses.

Identification of a newly recognized hantavirus from Yugoslavia

Hantaan (HTN), Puumala (PUU), and Seoul (SEO) viruses (family *Bunyaviridae*, genus *Hantavirus*) are recognized pathogens of humans. They produce severe, mild, or moderate clinical forms of hemorrhagic fever with renal syndrome (HFRS), particularly in Asia and Europe. Hantaan virus (which causes a severe form of HFRS) usually is isolated from field mice (*Apodemus* species), Puumala virus (which causes a milder form of HFRS known as Nephropathia Epidemica) usually is isolated from bank voles (*Clethrionomys glareolus*) in Scandinavian countries and many other European countries; both viruses are present in (what was) the European U.S.S.R. and in the Balkan countries. Seoul virus causes a less severe form of infection (Japan and Korea) than does Hantaan virus but a more severe form than does Puumala virus; Seoul virus is isolated from rats.

In 1961 an epidemic of HFRS occurred in Yugoslavia. Disease was mostly mild but a few cases were severe and one was fatal. The epidemic occurred in a group of 46 soldiers who apparently were infected when carrying out routine exercises in a forest 60 km west of Belgrade, Serbia. In 1967, a second epidemic occurred. This mostly affected forest workers and farmers in central Yugoslavia (Bosnia and Hercegovina, Croatia, and Montenegro). Cases were characterized by profuse bleeding and renal failure. In a third epidemic, which occurred in 1986 essentially throughout Yugoslavia, 161 clinical cases were serologically confirmed as being caused by hantaviruses; both mild and severe forms were recognized.

A fourth epidemic occurred in 1989, this one also with nationwide distribution. Using two serologic tests, immunodiffusion (IF) and enzyme immunoassay for detection of IgM antibody (IgM ELISA) and antigens of six different hantaviruses (HTN, PUU, SEO, Prospect Hill [PH], Fojnica [FOJ], and Vranica [VRA]; the latter being hantaviruses isolated in Yugoslavia), 226 clinical cases were confirmed as being caused by hantaviruses. In 135 of the 226 serologically-confirmed cases higher IF and ELISA antibody titers were found using antigens of HTN, FOJ and SEO viruses than using antigens of PUU, VRA, and PH viruses. The other 91 patients had higher antibody titers to PUU, VRA, and PH than to HTN, FOJ, and SEO viruses.

Patients with higher titers to HTN, FOJ, and SEO had two different kinds of antibody responses. By IF, some had about the same titers to HTN, FOJ, PUU, VRA, and SEO and lower titer to PH, but by ELISA they had strong reactions to HTN virus and no antibody to PUU virus. Another group of patients reacted much like those in Korea and China, having much higher antibody titers to HTN and SEO viruses and low titers to PUU and PH viruses by both serologic methods.

From erythrocytes and urine of patients with higher antibody titers to HTN, FOJ, and SEO viruses we isolated two different hantaviruses, which we called Kraljevo and Belgrade viruses. Kraljevo virus appears to be identical to FOJ virus, isolated from wild mice (*Apodemus flavicollis*) and closely related to the prototype HTN virus from Korea. The patient from whom blood and urine served as the source of Kraljevo virus developed a typical clinical course of severe HFRS, lapsed into coma on the fifth day after onset, and died three days later.

Three strains of Belgrade virus (Table 1) were isolated from blood and urine of HFRS patients, one of whom died 28 days after onset of symptoms and 12 days after lapsing into coma with pulmonary and kidney failure. Belgrade-1 and Belgrade-2 viruses are identical by IF, ELISA, plaque reduction neutralization, immunoenzyme neutralization, immune electronmicroscopy, and by biological characteristics in laboratory animals. They kill newborn mice and are distinct from HTN, PUU, SEO, FOJ, VRA, and PH viruses. Belgrade virus differs from Kraljevo virus and constitutes a newly recognized hantavirus serotype, for which we propose the name Belgrade virus.

(Submitted by: A. Gligic

Table 1. Cross-IFA and PRN antibody titers of hantavirus serotype-specific rat antisera.*

Rat immune serum	IFA titer							PRN titer						
	Hantaan			Prospect				Hantaan			Prospect			
	76-118	Fojnica	Krajlevo	Seoul	Puumala	Hill	Belgrade	76-118	Fojnica	Krajlevo	Seoul	Puumala	Hill	Belgrade
Hantaan	1024	1024	1024	512	512	<32	1024	1024	256	256	<32	<32	<32	<32
Fojnica	2048	>2048	2048	256	128	32	512	256	2048	2048	<32	<32	<32	<32
Kraljevo	1024	1024	>2048	256	128	32	1024	256	2048	>2048	<32	64	64	32
Seoul	512	512	512	2048	128	<32	128	<32	32	<32	1024	<32	ND	<32
Puumala	1024	2048	512	512	>2048	1024	512	<32	64	64	<32	1024	32	<32
Prospect Hill	64	128	32	<32	128	512	32	128	128	128	<32	32	512	<32
Belgrade	256	256	512	512	64	32	2048	<32	<32	32	<32	<32	<32	2048

*All sera, except that for Puumala virus, were from rats immunized with 100 pfu of hantavirus and bled 28 days later. The serum for Puumala virus was a convalescent-phase serum from a Swedish patient with nephropathia epidemica.

SUMMARY OF RECENT EXPERIMENTAL STUDIES ON CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS UNDERTAKEN AT THE PASTEUR INSTITUTE, DAKAR, SENEGAL.

Crimean-Congo haemorrhagic fever (CCHF) virus is a human pathogenic arbovirus transmitted by Ixodid ticks. Belonging to the CCHF serogroup, genus *Nairovirus*, family Bunyaviridae. CCHF virus is distributed over 3 continents in various ecosystems of sub-saharan Africa, southern and central Europe, central Asia and the Middle East. The virus has been isolated from a wide variety of vertebrates as well as numerous ticks, some of which have been shown to be efficient vectors. Nevertheless, the ecology of this virus still needs to be clarified, particularly in the Austral and western regions of Africa. During the past few years, we have undertaken various studies aiming to elucidate the role of certains vector ticks and vertebrate hosts in the natural cycle of CCHF virus. Various colleagues have indicated that they are unaware of certain studies. Thus, the following abstracts are intended to summarize these studies, which are in various stages of publication.

Transmission of CCHF Virus from Experimentally Infected Sheep to Hyalomma truncatum Ticks

Wilson M.L., Gonzalez J.P., Cornet J.P. & Camicas JL (1991) *Research in Virology*, 142, 395-404.

CCHF virus was inoculated into West African sheep that were simultaneously infested with adult *Hyalomma truncatum* ticks. The sheep developed a viremia and antibodies, indicating virus infection and replication; however, the length and magnitude of the viremia and serological response corresponded to the animal's immunological status. Tick attachment and feeding was not influenced by sheep infection. CCHF virus infection was aquired by 11-33% of female and 0-60% of male ticks. Infection in the ticks did not influence their feeding success as judged by weight at drop-off, and the weight of eggs produced by infected and non-infected ticks was similar. Transovarial transmission of CCHF virus was demonstrated in 2 of 12 (17%) egg batches from infected female ticks, but in none of 19 egg batches from ticks that tested negative for CCHF virus. Our results suggest that under certain ecological conditions, sheep may serve to amplify CCHF virus in nature through horizontal transmission and that the maintenance cycle also may be influenced by transovarial transmission to the next generation of ticks.

CCHF Virus Replication in Adult *Hyalomma truncatum* and *Amblyomma variegatum* Ticks

Gonzalez, J.P., Cornet J.P., Wilson M.L, & Camicas J.L. (1991). *Research in Virology*, 142, 483-488.

CCHF virus replication was studied in two potential tick vectors from West Africa. *Hyalomma truncatum* is an important tick species in CCHF virus ecology, and *Amblyomma variegatum* represents another common tick that feeds on a variety of hosts and is a potential vector. Ticks were infected by intra-anal inoculation. CCHF virus was reisolated 5 days p.i. from 100% of ticks tested by suckling mice inoculation. During the early phase of virus replication the virus titer increased in *H. truncatum* but not in *A. variegatum*. Virus titer increased gradually to more than 6 log LD50/ml at 100hrs. p.i. for *H. truncatum*, then slowly decreased on day 7 p.i. to a plateau until day 12.p.i.. Antigen was first detected in *H. truncatum* hemocytes 4 hours p.i. at a low level of cell-infection (≤1%); The rate of infected cells then increased through day 12 p.i.. CCHF virus titer in *A.variegatum* reached a plateau in 5 days, and remained at a moderate level of 2±.5 log LD50/ml up to 70 days p.i.. When we explored the distribution of the virus in *A. variegatum*, infection in the leg and hypostome hemolymph increase in a similar way through day 115 p.i.. Despite exhibiting a lower titer, *A. variegatum* still remained infected with CCHF virus at 4 months p.i. when tested by suckling mice inoculation. Long-term infection was seen in other studies on *H. truncatum* where virus was reisolated 11 months p.i. with a mean titer of 2.3 log LD50/ml (unpublished). The stability of CCHF virus titer after about 15 days of intrinsic incubation suggests that life-long, stable persitence of this virus may occur in certain suceptible adult ticks.

Sexual and Transovarian Transmission of CCHF Virus in *Hyalomma truncatum* Ticks

Gonzalez, J.P., Camicas, J.L., Cornet J.P. & Wilson M.L. (1992), *Research in Virology*, 143, 23-28.

Male *Hyalomma truncatum* ticks were inoculated with CCHF virus, hypostomectomized, and then allowed to mate with uninfected females feeding on a naive rabbit. After mating, CCHF virus was reisolated from 2 of 3 males tested and from 4 of 6 mated engorged females (titer≥ 2.2 log LD50/ml.). Vertical transmission was then demonstrated by virus reisolation from a portion of two of the six egg batchs laid by the positive females. From these 2 positive egg batchs, 6 larvae pools were tested, yielding virus reisolation from one. Attempts to reisolate CCHF virus from 15 nymph pools from this positive batch of larvae did not succeed.

Virus reisolation from gonopore-closed females which cofed with preinfected males, demonstrated transmission in the absence of copulation. Rabbits that served as blood meal sources seroconverted after infestation by infected male ticks. However virus was not reisolated from 3 gonopore-closed, engorged females, nor from their eggs, after a feeding with hypostomectomized preinfected males.

Transmission of CCHF virus during mating or cofeeding of adult *H. truncatum*., and subsequent transovarian transmission, appears to be another way to increase infection in the tick population, and may contribute to the maintenance of transmission in nature

Biological and Clinical Responses of West African Sheep to CCHF Virus Infection.

Gonzalez, J.P., Camicas, J.L., Cornet J.P. & Wilson M.L., *Research in Virology*, (submitted).

To further clarify the role of sheep in the maintenance cycle CCHF virus, we studied biological and clinical aspects of animals that were experimentaly infected with the virus.

A total of 17 sheep were infected either by intra-peritoneal inoculation or by infestation with experimentaly infected ticks. These adults, as well as 5 lambs born of infected females were monitored. Among clinical symptoms, only a moderate fever was observed (39.7°C±0.3) during the period of viremia. CCHF virus was reisolated after intracranial inoculation into suckling mice from blood samples taken on days 3 to 9 p.i. at a mean titer of 3.3 log LD50/ml.. Circulating virus was detected during 7 days in naive sheep and for less than 4 days in previously infected sheep. Antibody production was demonstrated by a direct ELISA (IgG) and by an IgM-capture ELISA. In non-immune sheep, IgM was first observed on day 7 p.i. and IgG appeared 1 day later. Among 5 sheep studied for liver and kidney functions, all showed hepatic dysfunction with a moderate elevation of serum aspartate transferase (210 U./l). Two of 4 sheep tested for blood responses had abnormal cell counts with a marked neutrophilia (up to 63%) lasting for two weeks begining at day 5p.i.. None of these changes in biological factors could not be directely related to CCHF virus infection. Ewes infected when pregnant ewes produced *post partum* antibodies in their milk at a significant titer (1/1,000) and antibodies were recovered in the sera of nursing lambs from the first meal to 50 days after birth with a decreasing titer.

Host Passage Induced-Phenotypic Changes in CCHF Virus.

Gonzalez, J.P., Camicas, J.L., Cornet J.P. & Wilson M.L., *Research in Virology*, (submitted).

The pathogenicity to suckling mice (SM) of intra-cerebrally CCHF virus has been used as a measureof virus' phenotype. We studied potential phenotypic changes associated with diffeent host passage histories. Two strains isolated from different hosts (human and tick) were passaged into other hosts (vertebrates and ticks) then reisolated and tested for pathogenicity in SM. More than 5.700 SM were inoculated with 13 viral supensions with different passage histories. Survival curves were established using the actuarial lifetime table and differences were evaluated with the log rank test. Regardless of the origin of the strain, viruses exhibited the same phenotype when passaged from mice to mice. However, the viral phenotype radically changed when another vertebrate host or a tick passage occured. The last host appears to be the major influence on SM pathogenicity. Because CCHF virus strains appear to vary little in their antigenic phenotype, perhaps hosts can induce phenotypic changes that modulate viral pathogenesis without producing detectable genotypic change.

Experimental Infections with CCHF Virus Using Laboratory Mice

Keyes L.E., Wilson M.L., & Gonzalez J.-P., *Research in Virology*, (submitted).

The serological, virological and pathogenic impact of Crimean-Congo hemorrhagic fever (CCHF) virus infection was studied using laboratory mice as a model. Mice of various ages and sexes were inoculated intracranially, intraperitoneally or subcutaneously with different virus titers. The effects of age, route of infection, and concentration of inoculum on viral pathogenicity and the development and transfer of viremia and antibody were investigated. Inoculation of day-old mice was similarly fatal at high titers, but the percentage of these mice that survived increased as the initial virus titer declined. Older mice better survived higher titer inoculation. Thus, we observed a dose-response-like pattern that was age-dependent. Adult mice which survived infection produced IgG beginning 3-5 post-inoculation. Antibodies of pregnant mice that were infected subcutaneously from 1 to 6 days prior to giving birth apparently were transferred to the infants who were not found to be viremic. Our results suggest that certain aspects of transmission and pathogenicity of this widespread, sometimes fatal, zoonosis may be studied in the laboratory by use of model vertebrates such as mice.

Temperature- and Humidity-Dependent Longevity of Unfed Adult *Hyalomma truncatum*.

Wilson, M.L., Dykstra, E.A., Schmidt, B.A., *Journal of Medical Entomology*, (submitted)

The survival of unfed adult *Hyalomma truncatum* Koch held under different regimes of constant temperature (5, 17, 24, 30 C) and relative humidity (RH) (10, 50, 80%) was monitored during >1yr. Longevity of this tick was shortest at the highest temperature and lowest RH (100% dead at week 25). Conversely, *H. truncatum* lived longest at lower temperatures and higher RH (<100% dead at week 64). The survival of males and females was similar and, curiously, was independent of the weight of ticks. These findings have implications for the maintenance and study of laboratory colonies of *H. truncatum*, and for the development of tick control strategies to reduce vectorial capacity.

Anyone wishing further information or reprints from the studies presented here should write or call us at : Yale Arbovirus Research Unit, Yale Univ., School of Medicine, P.O.Box 3333, New Haven, CT 06510 (USA); Fax: (203) 785-4782; tel.: (203) 785-2904/6110.

Report from: Jean-Paul Gonzalez and Mark L. Wilson.

Yale Arbovirus Research Unit, Yale University, New Haven, CT (USA) & Institut Français de Recherche scientifique pour le Développement en Coopération (ORSTOM).

ISOLATION OF *BORRELIA BURGDORFERI* FROM AN *IXODES DAMMINI* TICK COLLECTED IN PRINCE EDWARD ISLAND, CANADA

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Lyme disease has been reported in Canada with provisional data as of June, 1990 indicating that 103 cases have been identified from seven provinces[1]. Despite these reported cases, the only currently documented area of activity of *Borrelia burgdorferi* in Canada is at Long Point in southern Ontario. At this location, numerous isolates of *B. burgdorferi* have been obtained from ticks and mice in studies conducted from 1987 to 1990[2]. In 1989 three adult *I. dammini* were recovered from domestic pets in Prince Edward Island and four additional ticks were submitted to the Atlantic Veterinary College in 1990[3,4]. Therefore in 1991 a study was initiated to examine live collected ticks in Prince Edward Island for the possible presence of *B. burgdorferi*.

Seven live, engorged ticks were received for examination between October, 1991 and January, 1992. All were adult females of which three were removed from cats, three from dogs and one from a person's backpack. Five ticks were identified as *I. dammini* and two as *I. scapularis*. The pets off which the *I. dammini* were removed had no histories of recent travel whereas the dog off which the two *I. scapularis* were taken had recently travelled to the southern United States.

The gut contents of each tick were removed by use of a 2.5cc syringe and a 22 gauge needle. Each tick was surface sterilized by submerging in 95% ethanol followed by washing in sterile phosphate buffered saline. The needle was then inserted above the anal groove. Each syringe contained sterile BSKII medium of which approximately 0.1 mL was injected into the tick until it became fully distended. The medium was then removed along with the tick gut contents. Darkfield microscopy was performed on a drop of each tick suspension while the remaining material was inoculated into BSKII medium. The tubes were incubated at 33°C and monitored for growth by darkfield microsopy.

Darkfield microscopy of gut contents from an *I. dammini*, T27, removed from a cat in Charlottetown revealed the presence of numerous spirochetes. By two weeks postinoculation, the BSKII medium inoculated with T27 had given rise to a pure culture containing moderate growth of an isolate designated as T27-91. Antigen was prepared from isolate T27-91 and reacted by IFA with two monoclones kindly supplied by Dr. Alan Barbour, The University of Texas Health Science Center, San Antonio. Isolate T27-91 was

found to react both with monoclone H9724, a monoclone reactive with all Borrelia species, and monoclone H5332, a monoclone which reacts specifically with B. burgdorferi. Thus, isolate T27-91 was identified as B. burgdorferi.

To date 20 adult I. dammini have been documented on Prince Edward Island including one removed from a boy in Summerside in 1988[5] and 19 obtained between 1989-1991 primarily from domestic pets[3,4,6]. All of these ticks were obtained by passive surveillance and it is difficult to estimate how many ticks are actually being missed.

It is tempting to speculate that the I. dammini found in Prince Edward Island, including the one that yielded isolate T27-91, are being introduced by migrating birds coming from the Lyme endemic area on the eastern seaboard of the United States. Once introduced, the larvae or nymphs may develop into the adult stage that is currently being observed. The major adult host for I. dammini, the white tailed deer, does not occur naturally on Prince Edward Island, but the possible existance of alternative hosts is currently being explored.

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EXPOSURE TO BORRELIA BURGDORFERI (Bb) AND TICK-BORNE ENCEPHALITIS (TBE) VIRUS OF US TROOPS IN SOUTH-GERMANY

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In Europe, TBE-virus and Bb are present in the same reservoir rodent (mainly *Clethrionomys glareolus*), transmitted by the same tick (*Ixodes ricinus*) and may cause partially overlapping clinical-neurological symptoms.
A study was undertaken in 1989 to assess exposure to Bb and TBE-virus in military arrived since 1 to 3 years from the US (where TBE is not prevalent) and selected for intensive outdoor activities in Southern Germany, where both Lyme Disease and TBE are endemic. Paired sera were obtained from 511 subjects (mean age 25.9 y) before and after the summer. Antibodies against Bb or TBE were detected with EIA-systems, using a Bb B31 strain and a TBE Neudorfl-strain as respective antigens. Questionnaire data were used for epidemiological study.
IgG or IgM antibodies against Bb were found in one or both sera of 40 (7.8%) subjects. Of the non-immune subjects (n=489) in the first sample, 18 (3.7%) seroconverted during the study period. IgG antibodies against TBE-virus were found in 37 (7.2%) subjects and 14 (2.9%) out of 488 non-immune persons seroconverted. Three subjects had antibodies against both pathogens.
There was no significant correlation between Bb- or TBE-seropositivity or -seroconversion and different forms of outdoor activities, history of tick bite or typical clinical symptoms. Yellow fever vaccination status had no impact on TBE-seroprevalence.
In the population and in the geographic area under study, a striking parallelism was shown between Bb and TBE seroprevalence and -seroconversion rate, albeit without clinical implications. In Southern Germany, TBE may be warranted in the differential diagnosis of Lyme Disease.

CURRENT STATUS OF THE LYME BORRELIOSIS VECTOR, *IXODES DAMMINI*, IN MANITOBA

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The tick, *Ixodes dammini*, was discovered in the province of Manitoba for the first time in 1989. With numerous diagnosed cases of Lyme Borreliosis, and a high prevalence of antibodies reported in the human population, it was important to learn more about the distribution and abundance of *I. dammini*, the primary vector of the pathogen responsible. A vector survey was initiated, with the support of the Manitoba Health Research Council.

The survey was conducted during 14 May to 9 December, 1990, and 30 March to 29 June, 1991. Over 1600 small mammals were trapped and dragging samples collected in over 150 locations in southern Manitoba during the survey. No *I. dammini* were collected, except from a location near Marchand.

This area was first visited on 19-20 November, 1990. No adult ticks were collected during 15-5minute drags using a 1 m^2 flannel cloth. Sixteen small mammals were live-trapped (9 *Peromyscus maniculatus*; 6 *Clethrionomys gapperi*; 1 *Blarina brevicauda*), but none carried ticks. Two red squirrels (*Tamiasciurus hudsonicus*) were examined and no ticks were found.

In 1991, collections resumed at the Marchand sites on 16 May, when 6 animals were collected in 55 live-traps (1 *Spermophilus tridecemlineatus*; 2 *C. gapperi*; 2 *Eutamias minimus*; 1 *Microtus pennsylvanicus*). Larvae and nymphs of the American dog tick, *Dermacentor variabilis*, were removed from the two vole species, and a single *I. dammini* nymph was found on a red-backed vole.

During subsequent collections on 13 June (15 traps), and 29 June (55 traps), 3 (2 *M. pennsylvanicus*; 1 *P. maniculatus*) and 10 (3 *Tamias striatus*; 5 *C. gapperi*; 1 *M. pennsylvanicus*; 1 *P. maniculatus*) animals, respectively were collected. Nineteen larvae and 28 nymphs of *D. variabilis* were removed during the two collection periods, along with two *I. dammini* larvae, one each from *C. gapperi* and *P. maniculatus*. No tissue or blood samples were taken from any animals collected in 1991.

Previous collections of *I. dammini* consisted of single adult specimens only from each location. Intensive sampling effort by dragging and from collections of small mammals from these sites has been negative. Therefore, we concluded that these specimens were adventive, perhaps dispersing into Manitoba from endemic areas on migrating birds. However, the discovery of a nymph and two larvae from the same location near Marchand is reasonable evidence to suspect that a reproducing population of *I. dammini* exists there. Further sampling and attempts to culture *Borrelia burgdorferi* from ticks and small mammals are planned.

We would like to thank Drs. E.E. Lindquist and J.E. Keirans for confirming our tentative identifications of *I. dammini*.

LAST QUOTES

Russian proverb: "The shortage will be divided among the peasants."

Woody Allen: "For a while we pondered whether to take a vacation or get a divorce. We decided that a trip to Bermuda is over in two weeks, but a divorce is something you always have."

Woody Allen: "Cloquet hated reality but realized it was still the only place to get a good steak."

Woody Allen: "I was thrown out of college for cheating on a metaphysics exam; I looked into the soul of the boy next to me."

Woody Allen: "Not only is there no God, but try getting a plumber on weekends."

Bill Vaughan: "In the game of life it's a good idea to have a few early losses, which relieves one of the pressure of trying to maintain an undefeated season."

A. Bartlett Giamatti: "Baseball is about going home, and how hard it is to get there and how driven is our need. It tells us how good home is. Its wisdom says you can go home again but that you cannot stay. The journey must always start once more, the bat an oar over the shoulder, until there is an end to all journeying. Nostos: the going home, the game of nostalgia, so apt an image for our hunger that it hurts."

Craig Russell: "Canada is so square, even the female impersonators are women." (Sent by Harvey Artsob, Ottawa)

Erik Erikson: "In their search for a new sense of continuity and sameness, adolescents have to refight many of the battles of earlier years, even though to do so they must artificially appoint perfectly well-meaning people to play the roles of enemies."

Peter B. Medawar: "A virus is a piece of bad news wrapped in protein."

Clarence Darrow: "When I was a boy I was told that anybody could become President; I'm beginning to believe it."

La Rochefoucauld: "We all have the strength to endure the misfortunes of others."

Norman Maclean: "In our family there was no clear distinction between religion and fly fishing."

George Bernard Shaw: "A government which robs Peter to pay Paul can always depend on the support of Paul."

George Winters: "If God really intended men to fly, he'd make it easier to get to the airport."

Russian proverb: "The shortage will be divided among the peasants."

Dave Broadfoot: "Adam was a Canadian. Nobody but a Canadian would stand beside a naked woman and worry about an apple."

Alexander Woolcott: "I'm tired of hearing it said that democracy doesn't work. Of course it doesn't work. We are supposed to work it."

Unknown: "Join the army, see the world, meet interesting people, and kill them."

Samuel Johnson: "Americans are a race of convicts and ought to be thankful for anything we allow them short of hanging."

Arnold Toynbee: "America is a large friendly dog in a small room. Every time it wags its tail it knocks over a chair."

Robert Benchley: "A boy can learn a lot from a dog: obedience, loyalty, and the importance of turning around three times before lying down."

Garrison Keillor: "Cats are intended to teach us that not everything in nature has a function."

Jeff Valdez: "Cats are smarter than dogs. You can't get eight cats to pull a sled through snow."

Unknown: "The Jewish position on abortion is that a foetus is a foetus until it gets out of medical school."

Groucho Marx: "Go, and never darken my towels again."

Gloria Steinem: "Some of us are becoming the men we wanted to marry."

Luther Burbank: "Men should stop fighting among themselves and start fighting insects."

Unknown: "Time is nature's way of keeping everything from happening at once."

Fred Allen: "He writes so well he makes me feel like putting my quill back in my goose."

Ogden Nash: "Progress might have been all right once, but it has gone on too long."

Moses Hadas: "This book fills a much-needed gap."

Moses Hadas: "I have read your book and much like it."

Charles Pierce: "I would rather be black than gay because when you are black you do not have to tell your mother."

Will Rogers: "I don't make jokes. I just watch the government and report the facts."

Samuel Johnson: "A cucumber should be well-sliced, dressed with pepper and vinegar, and then thrown out."

Oscar Wilde: "The advantage of the emotions is that they lead us astray."

Unknown: "Its easy to make a friend. What's hard is to make a stranger."

Unknown: "The baby was so ugly they had to hang a pork chop around its neck to get the dog to play with it."

Derek Bok: "If you think education is expensive, try ignorance."

Jackie Mason: "I have enough money to last me the rest of my life, unless I buy something."

Redd Foxx: "Health nuts are going to feel stupid someday, lying in hospitals dying of nothing."

Unknown: "A gourmet restaurant in Cincinnati is one where you leave a tray on the table after you eat."

Lily Tomlin: "We're all in this alone."

Joseph Stalin: "Gaiety is the most outstanding feature of the Soviet Union."

Richard Lewis: "Most Texans think Hanukkah is some sort of duck call."

Groucho Marx: "It isn't necessary to have relatives in Kansas City to be unhappy."

Walter Bagehot: "The reason so few good books are written is that so few people who can write know anything."

Harry Truman: "I never did give anybody hell. I just told the truth and they thought it was hell."

Roy G. Blount, Jr.: "Doctors and lawyers must go to school for years and years, often with little sleep and with great sacrifice on the part of their first wives."

H.L. Mencken: "Every decent man is ashamed of the government he lives under."

Milan Kundera: "Right in the middle of Prague, Wenceslaus Square, there's this guy throwing up. And this other guy comes along, takes a look at him, shakes his head, and says, 'I know just what you mean.'"

Mark Twain: "If you tell the truth you don't have to remember anything."

H.L. Mencken: "The first Rotarian was the first man to call John the Baptist "Jack".

Ernesto Guevara: "Silence is argument carried on by other means."

Mahatma Ghandi (when asked what he thought of Western civilization): "I think it would be a good idea."

Susan Ertz: "Millions long for immortality who don't know what to do on a rainy Sunday afternoon."

Ronnie Shakes: "I like life. It's something to do."

Erma Bombeck: "Never lend your car to anyone to whom you have given birth."

Socrates: "Children today are tyrants. They contradict their parents, gobble their food, and tyrannize their teachers."

Arthur Baer : "Alimony is like buying oats for a dead horse."

Robert Orben: "Every morning I get up and look through the Forbes list of the richest people in America. If I'm not there, I go to work."

Nancy Reagan: "I believe people would be alive today if there were a death penalty."

Garrison Keillor: "They say such nice things about people at their funerals that it makes me sad to realize that I'm going to miss mine by just a few days."

Bernard Malamud: "We didn't starve, but we didn't eat chicken unless we were sick, or the chicken was."

Guindon cartoon caption: "Whatever other contributions to our society, lawyers could be an important source of protein."

David Ben Gurion: "Anyone who does not believe in miracles is not a realist."

Eleanor Roosevelt: "Nobody can make you feel inferior without your consent."

Edward R. Murrow: "Just because your voice reaches halfway around the world doesn't mean you are wiser than when it reached only to the end of the bar."

General Joseph Stillwell: "The higher a monkey climbs, the more you see of its behind."

Unknown: "Any new venture goes through the following stages: enthusiasm, complication, disillusionment, search for the guilty, punishment of the innocent, and decoration of those who did nothing."

Edmund Burke: "The only thing necessary for the triumph of evil is for good men to do nothing."

Professor Scott Elledge (on his retirement from Cornell): "It is time I stepped aside for a less experienced and less able man."