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ECOLOGY OF ARBOVIRUSES AND THEIR DISEASES IN FRENCH GUIANA

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Approaching the study of the Arbovirus in French Guiana we tried to make manifest the ecological foci, where by the only fact of the presence at the same time of virus reservoirs and of a population of vectors particularly abondant we had some chance of isolating some arbovirus which can cause a disease to man.

In order to identify these ecological foci we had to do a certain number of serological investigation trying to use man as a revelator of this arbovirus circulation. The serological investigation were executed first by using a series of antigen chosen because they have been isolated before in French Guiana :

- for the A group, Mucambo and Pixuna virus.
- for the B group, Yellow fever virus, St-Louis, Dengue II and Dengue III.

After the isolation of two virus : one, seeming new, belonging to the Venezuelan Encephalitis group, the other belonging to group B and recognized as similar to Ilheus, we have also included them in the series of antigens. Military coming from different territories and in particular from Martinica and Guadaloupe seemed to us to be excellent sentinel. In fact they had not had any previous contact with the circulating virus in Guiana or at least we believed so. Then again they were obliged to move around the territory and had thus all possibilities to be in contact with infected vectors.

It should prove that those coming from the Antilles (Martinica and Guadaloupe) has been mostly in contact with the Dengue and, being vaccinated immediately upon the territory against the yellow fever, should present, from the very beginning, secondary reaction of group B making it difficult, if not impossible, the manifestation of later contact with the virus of this group. We have then begun again the immunological investigation using no longer people from the Antilles but rather the soldiers coming from France for a staying of two years in French Guiana.

A taking of blood for a test was effected upon their arrival on the territory which showed the yellow fever vaccination they had submitted before leaving France and a second test was effected on the occasion they left the territory two years later.

This investigation which still goes on at this moment should indicate that the arbovirus circulation was in reality very insignificant at least with the series of antigens we use.

Prepared by Dr. Jean-Pierre Digoutte, Director, Institut Pasteur de la Guyane Française, Cayenne, French Guiana.

Other investigation, were similarly done in different villages situated in the entire territory.

They permitted to demonstrate the irregular distribution of group A virus and in particular of CA An 410 d(Tonate) along the coastal zone. The captures of vectors and those of birds and rodents were directed towards these ecological foci particularly favourable. Unfortunately all of them were not of easy access and in particular, we could not develop the captures the way we wished in the region of Kaw-Guisambourg where the carriers of antibodies for CA An 410 d (Tonate) are particularly numerous.

We shall consider successively each of isolated virus in Guyane insisting particularly on a new virus of the group of Venezuelan Encephalitis which was found, at one and the same time, in man, one of vertebrate hosts and numerous vectors.

DESCRIPTION OF THE REGION

a) CLIMATE.

French Guiana has an equatorial climate with two dry seasons and two wet seasons.

The longest dry season has a variable duration but it falls approximatively between August and December.

The smaller falls in February-March.

The longest wet season begins on April up to the beginning of August and the smaller wet season on December up to February.

Rain precipitation varies from one year to another and it ranges between 3 - 5 meters.

Trade-winds are predominant blowing regularly from North towards the East during wet season and from the East during the dry season.

One can distinguish several micro-climates which are connected in general with the topography, precipitation being increased in zones where the relief is more distorted.

Temperature is very stable. The average oscillates between 26 and 27°. Humidity is high and its average is superior to 85 %.

b) VEGETATION.

The greater part of the country is covered with the huge forest which, without interruption, extends over greater part of the Guianas and the Amazon basin.

The coastal belt having 3 to 20 kilometers of large is mostly savannah. At the west of Cayenne it is to be found dry savannah interrupted by gallery of forest.

At the east, on the other hand, the savannahs are under water during the major part of the year.

Between Kaw and Roura extends a vast swamp zone the sub-soil of which is formed with turf.

The majority of dry savannah are due to men's action who burn the grass several times a year.

At the interior of the country the huge forest varies in fonction of the constitution of the sub-soil.

The rocks are covered with stunted vegetation. The green rocks, lava and amphibolite are favorable to the growth of beautiful trees. On the other hand granites produce a puny and spare forest.

c) HYDROGRAPHY.

French Guiana is crossed from South to the North by many rivers and streams.

- On the East the Oyapock river which is the natural border with Brazil, then going towards the west the Approuague river, then, the Comte and the Orapu rivers, the streams of Kourou, of Sinnamary, that of Mana and finally,

- To the west, the Maroni river which is also the natural border with Suriname.

The island of Cayenne form a quadrilateral of 5 to 10 kilometers wide on 20 kilometers long situated between Mahury on the East, the Cayenne stream on the West, the Tour de l'Ile stream on the South which joins the Cayenne stream to the Mahury and the sea on the North.

Considering the vegetation stand point it is formed by a forest-savannah mosaïc owing to men's action.

The sensible relief is formed by the outcrop of ancient rocks and formed by mountains such as the Mahury plateau or the Mounts of Tigre reaching 150 or 170 meters of hight.

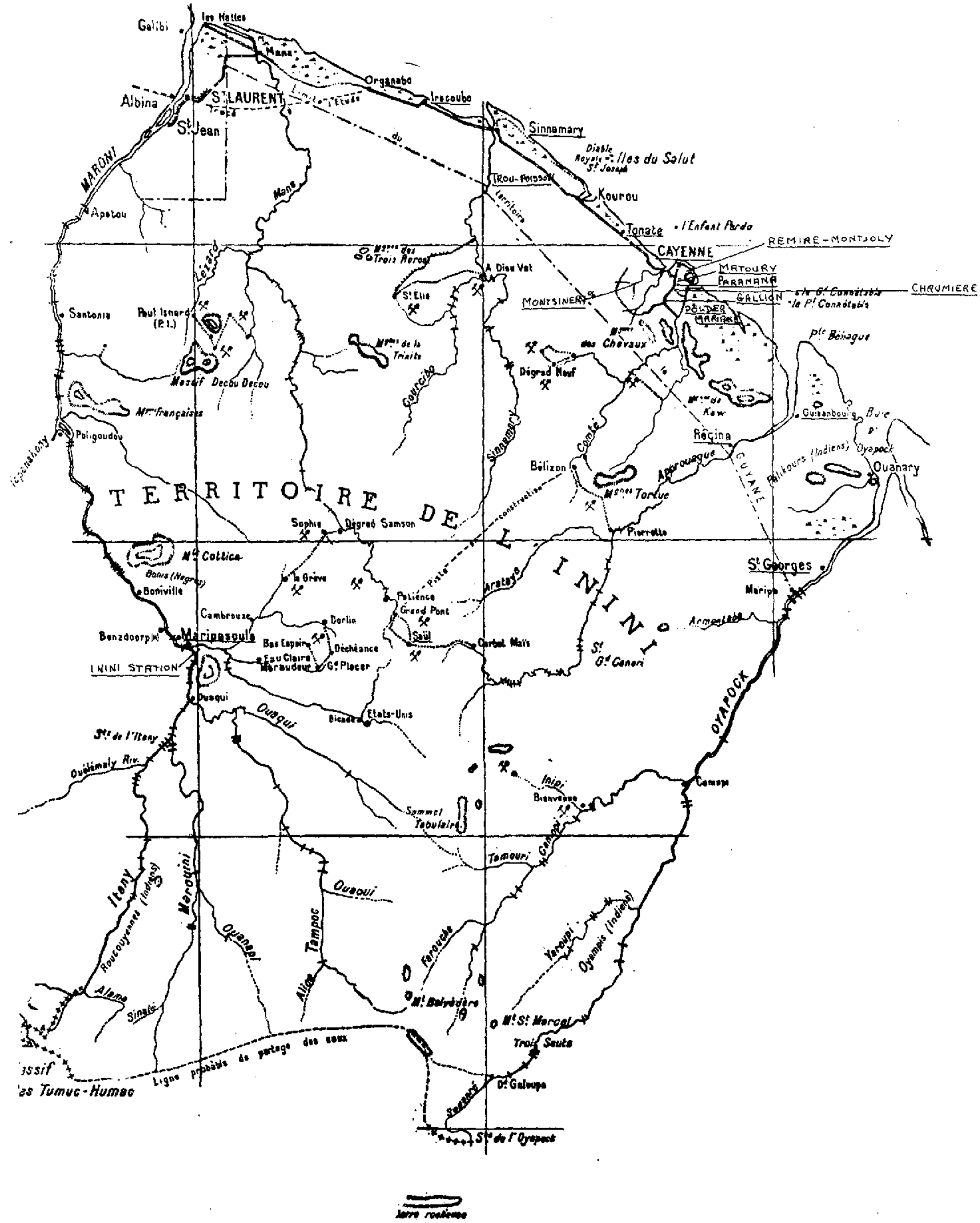
Between these mountains are often situated vast swamps.

The greater part of captures were taken along the coastal belt and most of it in a certain number of points of study situated not far from Cayenne.

The " Institut Pasteur " owns an experimental station, 15 kilometers far from Cayenne, named Paramana where most of the captures were effected.

In the interior of lands all the captures or serological investigation were done in the same experimental station situated on the Maroni at Maripasoula.

All the points of capture or immunological investigation can easily be found on the map (the places and village names are underlined).



Galibi
les Hattes
Mana
Organabo
Iracoubo
Albina
St. LAURENT
St. Jean
MARONI
Sinnamary
Iles du Salut
Kourou
Tonate
l'Enfant Perdu
CAYENNE
REMIRE-MONTJOLY
MATOURY
PARANANA
CHAUMIERE
GALLION
MONTSINERY
Apatou
Santonia
Massif Decou Decou
Mts Françaises
Poligoudou
St Elie
Dégrad Neuf
Approuague
Régina
Guisanbourg
Ouanary
Oyapock
Bélizon
Mgnes Tortue
Pierrette
TERRITOIRE DE L'ININI
Sophie
Dégrad Samson
Mt Cottica
Boniville
St Georges
Maripa
Cambrouze
Dorlin
Patience
Grand Pont
Saül
Carbet Maïs
Benzdoorp
Maripasoula
ININI STATION
Bas Espoir
Déchéance
Eau Claire
Maraudeur
Gd Placer
St Gd Caneri
Armontabo
Ouaqui
Etats-Unis
OYAPOCK
Inipi
Bienvenue
Camopi
Tabulaire
Tamouri
Itany
Marouini
Ouaqui
Tampoc
Mt Belvédère
Mt St Marcel
Trois Sauts
Alama
Massif
des Tumuc-Humac
Ligne probable de partage des eaux
Dt Galoupa
Sce de l'Oyapock
terre rocheuse

MATERIEL AND METHODS

Materiel of isolation

- MAN.

The serum for isolation of virus were taken as soons as possible after the beginning of the disease.

In general the blood was obtained in steriles tubes, allowed to clot at ambient temperature, centrifuged and inoculated immediately. The rest of the serum was divided into two vials one being stored at - 65°C and another in a freezer at - 25° C.

Each time it was possible a convalescent serum taken about three weeks after the beginning of the disease, was conserved in a freezer at - 25° C. 310 serums were inoculated.

- MOSQUITOES.

Mosquitoes are routinelly captured principally with human bait.

The teams of several capturers shift every two hours either, from 16 hours to 23 hours, or in a period of 24 hours. During the working hours each man was sat naked legs, and with the help of tubes take the mosquitoes which came to lie on him.

As soon as a mosquito is caught the tube was stopped with cotton.

The captures are equally realized with the help of portable light-traps type CDC.

After each period of captures the tubes holding the mosquitoes were placed in a ice box. At the end of the capture they were identified living in the tube of capture then grouped in monospecific lots in a polypropylene screw cap vials. They were placed directly in a freezer at - 70°C when the identifications were effected in the "Institut Pasteur" or put in a liquid nitrogen container then transfered to a freezer at -75°C upon arrival in the laboratory.

In 1973 and 1974 a total of 338.439 mosquitoes in 4 116 lots was collected and processed.

- VERTEBRATES.

The birds were either captured through a japanese nets or killed by a gun. The rodent and marsupial were captured with traps either locally wooden made or a standard metalic model. For each bird or rodent captured two or three samples were effected:

- 1 blood sample for isolation of virus a drop of blood was placed in a propylene tube containing suitable diluent Hanks + Bovalbumine 0,75 % + antibiotic Occ5.

- 1 sample of organs (brain, heart, spleen) which were placed all together in a polypropylene tube.

Whenever the size of the animal permitted, a blood sampling was harvested in order to search for antibodies in the serum. The samples were placed either in a nitrogen liquid container then in a freezer at - 75° C upon arrival at the "Institut Pasteur" or directly placed in the freezer when they were effected in the laboratory.

Samples were harvested on 3 045 wild birds and 170 rodents.

INOCULATIONS

- HUMAN SERUMS.

The serums were inoculated undiluted through mixed route : intra cerebral, subcutaneous and intra peritonal to a litter of suckling mice.

- ARTHROPODS

In a tissue grinder with a Teflon pestle, the mosquitoes were triturated with Hanks BSS added 0,75 % Bovin albumine and antibiotics,quantity was proportional to the number of mosquitoes and going from 0,5 ml for 1 to 3 mosquitoes at 6 ml for 100. The grinding of mosquitoes after centrifugation in a refrigerated apparatus was inoculated through mixed route(intra cerebral, subcutaneous and intra peritonal) to a litter of suckling mice.

- VERTEBRATE HOSTS.

The blood diluted at 1/10 in Hanks BSS was inoculated by mixed route at a litter of suckling mice.

In all cases for the human serum,the arthropods or the vertebrate hosts, at the time of the following passages,the animals were inoculated only i.c., one brain being grinded in a porcelar mortar and pestle with 2 ml diluant.

The reisolation were tried in the same technical conditions that the isolation.

METHOD OF IDENTIFICATION

The sensibility to chloroform was searched according to the FELDMANN and WANG technique.

The identification were done,immune ascitic fluid obtained with the Sarcome TG 180 strain, the mice undergoing 3 or 4 inoculations of virus.

The hemagglutination inhibition tests were practised according to CLARKE and CASALS(2) method working in micro plaque with sucrose acetone extracted antigen.

The same technique of preparation of antigen was used for the complement fixation test according to the L.B.C.F.(3) technique adapted by SEVER to the micro technique(4). The neutralisation test were practised placing on hour in water bath at 37° the mixture of the serum to be studied and the virus in successive dilution according to CAUSEY technique(5).

The neutralisation index is given by the difference between the titer of virus obtained in presence of serum to be tested and the titer obtained in presence of a serum without antibody.

The kinetic hemagglutination inhibition technique of CASALS(6) was used in order to compare the virus of the Venezuelian encephalitic group. It was used in its variant described by NATHANIEL A YOUNG(7) in which the test is adapted to the disposable plastic microplaque. The immune ascitic fluid treated with kaolin was allowed in contact with homologous and heterologous antigens during 0,5, two and 20 hours prior to the addition goose red cells. The antigens and ascitic fluids were diluted in tubes before being dropped under the volume of 0.025 ml in each well.

Because some of strain semms to be antigenically far, instead to test four dilution of immune fluid wa have tested every dilution with 3 antigens dilutions containing respectively 4, 8 and 16 hemagglutining unit.

RESULTS AND DISCUSSION

I - VIRUS ISOLATION.

1) Mucambo virus.

This virus was isolated only once in November 1972 of a vector genus Lutzomyia captured in Montsinery a small village 30 kilometers far from Cayenne.

On the other hand, on March 1973, we found it again four different occasions within birds organs captured in Maripasoula 300 kilometers far from Cayenne in the middle of the equatorial jungle (Turdus nudigenis, Tachyphonus cristatus, Trogon violaceus, Monasa atra).

At the moment these isolations took place this virus was not manipulated in the laboratory.

On February 1973 Mucambo was isolated twice from two laboratory workers who had manipulated the strain of reference. Again we isolated it on May-June 1973, from two other members of personnal who, similary had manipulated the strain of reference.

In all cases the symptomatology was very stereotyped showing through a h high fever, extremely brutal appearance with myalgias, head ache, nauseas or vomiting accompanied with a very high asthenia lasting for about 15 days.

From February 1973 this virus has never been found from the vector or host vertebrate.

All the serological investigations made, show that antibodies are found again at very low rates not exceeding never 1/10 or 1/20 in hemagglutination inhibition (HI) or 1/8 in complement fixation (CF), for Mucambo they always accompany, whether it be in CF reaction or HI test, antibodies at a very high titer for CA An 410 d (Tonate) which we shall study hereafter.

It does not seem consequently that this virus interfere now in the human pathology.

2) CA An 410 d (Tonate).

a) Isolation of Prototype strain.

On January 16 1973 in Tonate, a small village situated about 30 kilometers at the North-West of Cayenne on the road along the coast and leading to the Spatial Center of Kourou a Psarocolius decumanus was captured.

As usual two samples were taken : one from the organs (brain, heart, spleen) one from the blood (one drop with 0cc5 of Hanks with 0,75 % of Bovalbumine).

The two sampling were inoculated at two litter of suckling mice.

The suckling mice inoculated with organs showed a paralysis within 48 hours, a first passage was effected on one litter; the average survival time reduced to thirty six hours.

b) Identification.

The virus gives an hemagglutinin after sucrose acetone extraction.

The titer is 1/10.000 at the Ph optimum of 5,75-6.0 and at the three temperature (37° 4° R.T.).

The same antigen is compared in CF test with all the reference immune ascitic fluids we have. The reaction is negative with every one except with Mucambo and Pixuna.

In quantitative reaction the titer of the immune ascitic fluid Mucambo is 1/4 and that of the ascite immune Pixuna is 1/8, the optimum titer of the antigens beeing 1/16.

A mouse immune ascitic fluid is prepared from this strain which is compared again in complement fixation reaction with Mucambo and Pixuna.(Table I).

TABLE I

COMPLEMENT FIXATION TEST
CROSS REACTION MUCAMBO, PIXUNA, CA An 410 d

Antigens	Immune Ascitic fluid		
	CA An 410 d	Mucambo	Pixuna
CA An 410 d	64/32	8/16	< 8/8
Mucambo	16/4	32/8	8/16
Pixuna	< 8/8	< 8/8	128/128

The results show that CA An 410 d is very clearly different from Mucambo and Pixuna. One neutralisation test confirmed that CA An 410 d and Mucambo are different (Table II).

TABLE II

NEUTRALISATION-TEST

CROSS REACTION MUCAMBO- CA An 410 d

Virus	Non immune serum	Immune ascitic fluid	
		410 d	Mucambo
CA An 410 d			
Titer	9	3.5	7.5
Neutralisation Index		5.5	1.5
Mucambo			
Titer	8.5	5.5	3.8
Neutralisation Index		3	4.7

c) Isolation from Mosquitoes.

The CA An 410 d virus was isolated many times from March 1973 :

- A first isolation CA Ar 15268 was done, proceeding from Culex portesi captured at the Experimental Station of the Paramana near Cayenne on March 30 1973.

This strain was reisolated without difficulties.

- A second strain CA Ar 15391 was isolated from batch of 102 Culex portesi caught the same place on April 26 1973.

This strain was equally reisolated without difficulties.

- A third strain CA An 15430 was isolated from Culex portesi caught at "Camp du Gallion" situated in the same ecological context approximately 10 kilometers from Paramana.

On May 1973, a strain, CA Ar 15360 was isolated from batch of 60 Culex portesi captured at Paramana.

On December 1973 two others strains CA Ar 16623 and CA Ar 16647 were isolated from two batchs of Culex portesi caught at "Camp du Gallion" and at Stoupan, a small village situated at the same ecological site as the "Camp du Gallion"and the Paramana.

As an example the results of identification of the strain CA Ar 16647 in complement fixation test are given in the following Table III.

TABLE III

COMPLEMENT FIXATION TEST

CROSS REACTION CA Ar 16647 - CA An 410 d - CA Ar 508 - MUCAMBO - PIXUNA

Antigens	IMMUNES ASCITIC FLUIDS				
	CA Ar 11647	CA An 410 d	CA Ar 508	MUCAMBO	PIXUNA
CA Ar 16647	256/128 *	64/32	<8/8	<8/8	<8/8
CA An 410 d	256/128	32/32	<8/8	<8/8	<8/8
CA Ar 508	32/32	<8/8	128/16	<8/8	<8/8
Mucambo	16/64	<8/2	<8/2	16/32	<8/2
Pixuna	<8/8	<8/8	<8/8	<8/8	256/128

* Maximum titer of Ascitic Fluid/Optimum titer of antigen.

On December 1974 a strain , CA Ar 18612, was isolated of a batch of 100 Culex portesi caught at the "Camp du Gallion".

The antigen prepared by sucrose acetone extraction gives a negative reaction with every immune ascitic fluid except that prepared with CA An 410 d. The immune ascitic fluid prepared with this strain gives a positive reaction with CA An 410 d and CA Ar 508. The cross reaction is given in Table IV.

TABLE IV

COMPLEMENT FIXATION TEST
CROSS REACTION CA Ar 18612 - CA An 410 d - CA Ar 508

Antigens	IMMUNES ASCITIC FLUID		
	CA Ar 18612	CA An 410 d	CA Ar 508
CA Ar 18612	128/64 *	256/32	<8/8
CA An 410 d	128/64	128/32	<8/8
CA Ar 508	8/32	8/32	256/16

* Maximum titer of Ascitic Fluid/Optimum titer of antigen.

On September 1974, a strain CA Ar 18663 was isolated of 100 Mansonia titillans caught at Trou-Poisson a village situated some 150 kilometers West of Cayenne on the road crossing the coastal savannah.

From October 1974 to February 1975 18 strains were isolated from different kinds of mosquitoes.

The results are summurised in the following table :

TABLE V

STRAINS OF CA AN 410 d ISOLATED FROM MOSQUITOES

(October 1974 to February 1975)

Date	N° of batch	Type of mosquitoes	Number	Place of capture
30/X/ /74	CA Ar 18876	Culex portesi	100	Paramana
31/X /74	CA Ar 18861	Culex portesi	100	Paramana
31/X /74	CA Ar 18882	Culex portesi	100	Paramana
31/X /74	CA Ar 18883	Culex portesi	100	Paramana
6/XI /74	CA Ar 18917	Culex portesi	100	Matoury
6/XI/ 74	CA Ar 18920	Culex portesi	100	Matoury
6/XI/74	CA Ar 18932	Wyeomyia melanocephala	63	Matoury
14/XI/74	CA Ar 19004	Culex portesi	100	Gallion
15/XI /74	CA Ar 19029	Culex portesi	100	Paramana
25/XI /74	CA Ar 19100	Culex portesi	100	Paramana
25/XI /74	CA Ar 19101	Culex portesi	100	Paramana
4/XII/74	CA Ar 19151	Culex portesi	100	Gallion
12/XII/74	CA Ar 19263	Culex portesi	100	Chaumière
27/XII/74	CA Ar 19360	Culex spissipes	100	Trou-Poisson
9/I /75	CA Ar 19401	Wyeomyia melanocephala	6	Cayenne (Baduel)
14/I /75	CA Ar 19466	Wyeomyia occulta	2	Cayenne (Chatenay)
8/II /75	CA Ar 19678	Culex zeteki	13	Chaumière
19/II /75	CA Ar 19782	Culex portesi	100	Gallion

d) Isolation from man.

On July 1973 during a prospection carried out in the Iracoubo area a small village situated 150 kilometers North-East from Cayenne, a blood sample was harvested from a patient showing a febrile illness with cephalalgia. A blood smear taken same day showed the presence of Plasmodium falciparum.

After inoculation to a litter of suckling mice a strain CA H 73-379 was isolated and reisolated without difficulty from the serum of the patient. This strain has a short incubation since the average survival time passed from three days at the isolation to 24 hours after the first passage.

It is pathogenic for the suckling mice and for the mouse twenty one days old by intra cerebral way and no pathogenic for the adult mouse by intra peritoneal way on suckling mice the titer is $10^{8.5}$ by intra cerebral(i.c.) route. It gives a good hemagglutinin at Ph.6.0-6.2 after sucrose acetone extraction.

A convalescent serum could be obtained 40 days after the beginning of the illness. The acute and convalescent serums was tested in hemagglutination inhibition reaction and in complement fixation test with the antigens that we always use; we added to the series, the antigen CA H 73-379 used for the complement fixation test at the arbitrary dilution of 1/8.

The results are given in Table VI.

TABLE VI

HEMAGGLUTINATION INHIBITION AND COMPLEMENT FIXATION TEST

OF THE ACUTE AND CONVALESCENT SERA

Antigens	Acute serum		Convalescent serum	
	IHA	CF	IHA	CF
Mucambo	<10	<8	<10	<8
Pixuna	<10	<8	<10	<8
CA H 73-379	<10	<8	320	16
Yellow fever	<10	<8	<10	<8
Dengue II	<10	<8	<10	<8
Dengue III	<10	<8	<10	<8
Ilheus	<10	<8	<10	<8

The acute and convalescent serums diluted from 1/2 to 1/64 were tested again in CF test in presence of Pixuna, CA Ar 4389, CA An 410 d and CA H 73-379 diluted from 1/8 to 1/64 and in presence of Mucambo diluted from 1/2 to 1/16.

The results are given in Table VII.

TABLE VII

COMPLEMENT FIXATION TEST

CROSS REACTION ACUTE AND CONVALESCENT SERA OF THE PATIENT

WITH ANTIGENS MUCAMBO - PIXUNA - CA Ar 4389 - CA An 410 d - CA H 73-379

Antigens	Acute serum	Convalescent serum
Mucambo	< 8/2	< 8/2
Pixuna	< 8/8	< 8/8
CA Ar 4389	< 8/8	< 8/8
CA An 410 d	< 8/8	64/32 *
CA H 73-379	< 8/8	64/32

* Maximum titer of serum/optimum-titer of antigen.

The antigen CA H 73-379, at 1/8 was compared by complement fixation test with all the immune ascitic fluids of reference diluted 1/8. The reaction is positive with CA An 410 d and CA Ar 4389 and negative with the others.

Immune ascitic fluid was prepared with this strain and compared in cross reaction of complément fixation with the reference virus of the VEE group.

The results are given in Table VIII.

TABLE VIII

COMPLEMENT FIXATION TEST

CROSS REACTION STRAIN CA H 73-379 WITH OTHERS VIRUS OF VEE GROUP

Antigens	Immunes ascitic fluid				
	CA H 73-379	CA An 410 d	CA Ar 508	Mucambo	Pixuna
CA H 73-379	64/32	32/32	< 8/8	8/32	8/32
CA An 410 d	128/16	32/16	< 8/8	< 8/2	< 8/8
CA Ar 508	8/16	8/16	64/8	8/8	8/8
Mucambo	8/4	16/4	< 8/8	32/8	8/16
Pixuna	< 8/8	< 8/8	< 8/8	< 8/8	128/128

This strain seems then to be identical to CA An 410 d and different from Mucambo, Pixuna and CA Ar 508.

e) Results of serological investigations in man.

After the isolation of the CA An 410 d a strain from a patient serum, this one was included in the panel of antigen used for testing the human serums either from patient or from individuals living in different points of the territory.

The results are given in Table IX.

TABLE IX

COMPLEMENT FIXATION AND INHIBITION OF HEMAGGLUTINATION TEST SERA FROM MEN OF FRENCH GUIANA TESTED WITH MUCAMBO- PIXUNA- CA An 410 d

Place	N° sera	N° positive sera % positive	INHIBITION OF HEMAGGLUTINATION			COMPLEMENT FIXATION TEST		
			Mucambo	Pixuna	CA An 410d	Mucambo	Pixuna	CA An 410 d
Cayenne	286	32	10	10	320	–	8	8
			–	–	80	–	–	–
			80	80	80	–	–	–
			20	20	80	–	–	–
			20	20	640	–	–	–
			–	–	40	–	–	–
			–	–	40	–	–	–
		11 %	40	–	160	–	–	8
			160	20	640	–	–	–
			40	40	80	–	–	–
			–	–	320	–	–	–
			80	80	320	–	–	–
			–	–	80	–	–	–
			–	–	160	–	–	–
			160	80	640	8	8	32
			640	160	640	8	8	16
			10	10	160	–	–	–
			20	20	160	–	–	8
			10	10	80	–	–	8
			20	10	80	–	–	8
			320	80	640	8	8	16
			80	80	320	–	–	16
			80	40	320	–	–	8
			20	20	320	–	–	–
			40	80	320	–	–	–
			80	40	320	–	–	8
			10	10	160	–	–	–
			10	10	160	–	–	–
			160	80	320	8	8	16
			10	10	40	8	8	8
			–	–	160	–	–	–
			80	80	640	–	–	16

TABLE IX (Continuation)

Place	N° SERA	N° positive sera % positive	INHIBITION OF HEMAGGLUTINATION			COMPLEMENT FIXATION TEST		
			Mucambo	Pixuna	CA An 410 d	Mucambo	Pixuna	CA An 410 d
St-Georges	53	15	160	320	80	–	–	8
			–	–	640	8	8	32
			–	–	40	–	–	–
			–	–	20	–	–	–
			–	–	80	–	–	–
			–	–	640	8	8	16
			–	–	40	–	–	–
			20	80	160	–	–	8
		28 %	–	–	40	–	–	–
			40	80	160	–	–	8
			–	–	40	–	–	–
			20	40	80	–	–	8
			–	–	40	–	–	8
			–	–	40	–	–	8
			–	–	40	–	–	–
Rémire	47	7	40	160	640	8	8	16
			40	320	640	–	–	16
			10	80	80	–	–	8
			20	20	40	–	–	–
		15 %	–	–	40	–	–	–
			10	–	40	–	–	–
			40	40	40	–	8	8
Sinnamary	44	0						
		0 %						
Saül	52	5	–	–	40	–	–	–
			20	80	80	–	8	8
			10	10	20	–	–	–
		10 %	–	–	20	–	–	–
			40	40	80	–	–	–
Régina	37	13	–	20	160	–	–	8
Kaw			20	40	320	8	8	16
Guisambourg			10	10	160	–	–	8
			–	–	320	–	–	8
			–	–	80	–	–	8
			–	–	80	–	–	8
		35 %	–	–	20	–	–	–
			–	–	20	–	–	–
			10	20	80	–	–	8
			–	–	80	–	–	8
			–	–	80	–	–	8
			10	20	160	–	–	8
			20	20	320	–	8	16
Montjoly	14	0	0					
		0 %						
Matoury	42	10	40	40	640	–	8	8
			–	–	20	–	–	–
			80	80	320	8	8	16
			10	20	320	–	–	8
			20	20	320	–	–	8
			–	–	320	–	–	8
		24 %	–	–	160	–	–	8
			–	–	20	–	–	8
			10	10	160	–	–	8
			40	40	320	–	–	8

3) CA Ar 508.

This virus has been isolated twice with in three years, it was moreover identifying the first isolation, CA Ar 4389 , that we have verified, first, that this strain belonged to the group but was different from all those we had as reference strain, and secondly that it was similar to CA Ar 508 isolated by Ch.SERIE and considered so far as Pixuna.

CA Ar 4389 has been isolated on October 17 1972 from Culex portesi caught at Paramana Station, this strain was quickly adapted and the average survival time was stabilized to 24 hours as early as the first passage.

It gives an hemagglutinine and presents the reactions to chemical and physical agents of an arbovirus.

Compared to all strains of reference that we had on May 1973 in complement fixation test it gives a positive reaction with Mucambo, Pixuna and CA An 410 d.

The cross reaction are given in Table X.

TABLE X

COMPLEMENT FIXATION TEST
CROSS REACTION CA Ar 4389 - CA An 410 d- Mucambo- Pixuna

	IMMUNES ASCITIC FLUIDS			
	CA Ar 4389	CA An 410 d	MUCAMBO	PIXUNA
CA Ar 4389	64/64	16/128	8/64	8/16
CA An 410 d	32/64	64/64	8/64	8/16
Mucambo	16/16	16/8	32/8	8/16
Pixuna	8/64	8/64	8/8	128/32

During the period of training at Yale Arbovirus Research Unit, Geneviève PANON, Chief of Laboratory of the "Institut Pasteur " compared this strain to the VEE virus group.

The results are given in Table XI.

TABLE XI

COMPLEMENT FIXATION TEST (YALE ARBOVIRUS RESEARCH UNIT)

CROSS REACTION CA Ar 4389 - MUCAMBO - PIXUNA - V E E

	IMMUNES ASCITIC FLUIDS			
	CA Ar 4389	MUCAMBO	PIXUNA	V E E
CA Ar 4389	128/256	0/0	64/512	32/512
Mucambo	8/32	8/128	-	-
Pixuna	8/32	-	512/128	-
VEE	8/128	-	-	128/512

The reactions show that CA Ar 4389 is different from Mucambo, Pixuna and VEE.

We have then used again the strain CA Ar 508 isolated by Charles SERIE in 1968 to compare it with the strain CA Ar 4389.

The results are given in Table XII.

TABLE XII

COMPLEMENT FIXATION TEST

CROSS REACTION CA Ar 4389 - CA Ar 508 - CA An 410 d - MUCAMBO - PIXUNA

	IMMUNES ASCITIC FLUIDS				
	CA Ar 4389	CA Ar 508	CA An 410 d	MUCAMBO	PIXUNA
CA Ar 4389	64/32	128/16	16/16	8/32	8/16
CA An 508	64/16	28/16	8/32	8/8	8/8
CA An 410d	32/32	<8/8	64/32	8/16	<8/8
Mucambo	8/8	<8/2	16/4	32/8	8/16
Pixuna	8/64	<8/8	<8/8	<8/8	128/64

These reactions prove that CA Ar 4389 is similar to CA Ar 508 but it is different from the strains of the VEE group which we have : CA An 410 d, Mucambo and Pixuna.

No other strain of virus CA Ar 508 has been isolated from October 1972 to November 1974.

On November 1974, from a lot of 100 Culex portesi captured at Gallion a strain CAAr 19007 was isolated. This strain was to prove similar to CA Ar 508.

As we show in the Table XIII.

TABLE XIII

COMPLEMENT FIXATION TEST

CROSS REACTION CA An 410 d - CA Ar 508 - MUCAMBO- PIXUNA - CA Ar 19007

ANTIGENS	IMMUNES ASCITIC FLUIDS				
	CA An 410 d	CA Ar 508	CA Ar 19007	MUCAMBO	PIXUNA
CA An 410 d	256/64	<8/8	64/16	<8/2	<8/2
CA Ar 508	16/16	128/16	512/16	8/8	8/8
CA Ar 19007	16/32	128/32	512/64	<8/2	<8/2
Mucambo	32/32	<8/2	16/8	128/16	<8/2
Pixuna	16/32	<8/8	32/32	<8/8	64/32

4) UNA.

Two strains of Una virus were isolated on May and one on August 1973. The first from Psorophora ferox caught in Montjoly near Cayenne, the second from Coquillettidia venezuelensis caught in Sinnamary the third from Culex albicosta caught in Paramana station.

5) DENGUE.

The last outbreak of Dengue in French Guiana happened in February 1970.

During this epidemic three strain of Dengue II had been isolated by Charles SERIE from patient serum. Further more, a certain number of serological conversions of primary or second type were observed. This epidemic seems to be finished on July 1970.

From the beginning of the year 1973 all the military men coming from France submitted to a taking of blood for testing upon their arrival on the territory and then a second one when they are leaving the territory. No serological conversion for the Dengue nor for any other arbovirus was observed.

No isolation of Dengue was done, nor any serological conversion observed from serum of patients . It does not seem then there is now virus circulation.

6) ILHEUS.

a) Isolation from vertebrate host.

The first isolation of the virus was made from of blood of a bird Leistes militaris captured on February 1973 in Montsinery. This strain did not exist in the "Institut Pasteur" before this.

On April 1973 two other virus were recovered from organs of bird not identified and from Piaya minuta captured in Iracoubo and then a last isolation was made from the blood of Momotus momota captured in Maripasoula.

For all these isolations average survival time shortened progressively as of the passages it seems then that these isolation be authentic.

b) Isolation from man.

On March 1973 two strains were isolated from the blood of a patient who had just been admitted to the Hospital in Cayenne.

This patient presented on his entering the hospital a febrile condition with cephalalgia and shiver. The examination of a blood smear showed the presence of plasmodium falciparum. A taking of blood was made and after , having been decanted, the serum was inoculated, on one part by intra cerebral(i.c.)route to suckling mice, and on the other part by intra thoracic route at three lots of 12 Aedes aegypti from breading.

The first strain CA H 73-93 was isolated from sick suckling mice after 6 days of observation. The average survival time was four days after the third passage.

The three lots of Aedes aegypti were kept in observation during 10 days 25 survivors were gathered and inoculated to litter of suckling mice.

A strain CA 15289 produced illness and death in 4 days.

Acute and convalescent serums of the patient were tested by HI and CF in presence of virus we dispose and of two strains CA H 73-93 and CA 15289.

The results are given in the Table XIV.

TABLE XIV

HEMAGGLUTINATION INHIBITION AND COMPLEMENT FIXATION TEST OF THE ACUTE AND CONVALESCENT SERA

	Acute serum		Convalescent serum	
	IHA	CF	IHA	CF
Mucambo	<10	<8	<10	<8
Pixuna	<10	<8	<10	<8
Yellow fever	<40	<8	80	<8
S L E	<10	<8	80	<8
Dengue II	<10	<8	40	<8
Dengue III	<10	<8	20	<8
CA H 7393	<10	<8	640	16
CA 15289	<10	<8	640	16

The tests made in Cayenne showed that this strain belonged to the group B but it was different of those we had.

Sent to Yale Arbovirus Research Unit was recognized as very close if not similar to Ilheus.

7) MURUTUCU.

a) Isolation of vector.

8 strains of virus Murutucu were isolated from 1972 to 1975 :

- 7 from Culex portesi all captured in an area near Cayenne
- 1 from Anopheles peryassui captured at the "Camp du Gallion".

b) Isolation in man.

On November 1973 a patient showing a febrile syndrome with cephalalgia and myalgia is sent to the hospital in Cayenne.

His serum is inoculated i.c. into to group of six one day mice and at two lots of 10 Aedes aegypti by intra thoracic route.

After 10 days observation the two lots of Aedes aegypti are inoculated suckling mices which present a paralysis immediately after 36 hours.

The hemagglutinin prepared from livers of mice is inhibited by the convalescent serum of the patient.

This strain was recognized as close, if not,similar to Murutucu.

8) GUAMA GROUP.

19 virus reacting in complement fixation test with immune ascitic fluid Guama, Bimiti and Catu were isolated :

- 16 from Culex portesi
- 1 from Culex spissipes
- 1 from Coquillettidia venezuelensis
- 1 from Culex sp.

A strain of the same group CA An 265 d was isolated and reisolated from organs of bird Galbula dea.

This group, for the time, being is not object of deep studies and the Guama virus, Bimiti and Catu not being able to be separated by simple complement fixation test it is impossible for us to know the virus in question are.

9) TEMPORARY NEW VIRUS.

Four virus don't give any cross reaction with the antigens of reference wa have. One has been isolated from a bird, the others from vectors. One among them was isolated too from the serum of a patient died of hepatitis.

- CA An 1093 a.

This virus was isolated from the blood of a bird Pteroglossus aracari captured on September 1973 at Inini ground-station.

The suckling mice inoculated i.c. died on the day three post inoculation immediately after the second passage. This strain is sensible to chloroform and filters through a millipore membrane 0,22 µ.

- CA Ar 16102.

Isolated from 100 Culex portesi captured on September 3 1973 in Paramana station, this strain is adapted with difficulty,not less than twelve passages were necessary to obtain a regular mortality of suckling mice.

- CA Ar 16468.

a) Isolation from vectors.

This strain was isolated and reisolated from 55 Aedes serratus captured on November 1973 at Inini ground station in Maripasoula. It is sensitive to the chloroform, filters through millipore membrane 0,22 µ,and gives an hemagglutinin of weak titer 1/80 with only Ph of 6.2.

It was found again in a lot of 100 Mansonia titillans at "Polder Marianne" on November 1973.

b) Isolation from the blood of a patient.

A strain 74-635 was isolated on July 1974 from the blood of a patient (woman) very febrile and admitted to the Reanimation department of the Hospital having a heavy hepatitis. This patient died a few days after, the virus not being able to be authentified. However, it is the only isolation of this strain which was realized at this time, it was not manipulated neither in the laboratory where the reference strains are kept, nor in the laboratory where isolating process is practised. These two laboratories by the way are distant one from the other. It seems then this virus is authentic.

In complement fixation test this strain gives a positive reaction with CA Ar 16468, the cross reactions (Table XV) proves that it is similar to it.

TABLE XV

COMPLEMENT FIXATION TEST

CROSS REACTION CA Ar 16468 - CA H 74-635

Antigens	IMMUNE ASCITIC FLUIDS	
	CA H 74-635	CA Ar 16468
CA H 74-635	128/16	64/16
CA Ar 16468	128/32	64/32

- CA Ar 16652.

This virus was isolated from a lot of 18 Anopheles peryassui captured in "Gallion" on November 1973.

In complement fixation test it is different from all the strains of reference we have.

These four strains considered as temporarily new were adressed to the Yale Arbovirus Research Unit.

COMMENTARIES

THE GROUP A VIRUSES

SHOPE and collaborators in 1964 (8) describe two new prototypes of virus which are isolated in Brazil and seeming sufficiently different, according to the standard serological tests, from the reference strains VEE to consider them as new virus. He calls them Pixuna and Mucambo.

These two agents entered in the VEE complex. The isolation of the prototype strain was realized from a sentinel monkey Cebus appela . This virus was found again many times in the rodents and marsupials in Brazil as well as in Trinidad (9).

The most frequent vector is Culex portesi which permitted the isolation of many strains in Brazil, Trinidad and French Guiana(10).

Some virus isolations were made more rarely from other species of Culex and equally from Aedes, Mansonia, Haemagogus, Sabethini and Wyeomyia.

In Cayenne the last isolation from a vector was made on November 1972 from a pool of Lutzomyia genus.

Mucambo was isolated only once from a bird Pipra erythrocephala in province of Para, Brazil(11). On March 1973 we isolated it four times from four birds captured the same day, the same place, at the experimental station of Maripasoula in the middle of the forest.

It seems that this virus does not circulate anywise in the area we actually prospect and was replaced by CA An 410 d(Tonate) which belongs to the same group but can be easily differenciated by the classic complement fixation and neutralization test. The pathogenicity of this virus is very near that of Mucambo. It is pathogenic for the suckling mice and for the weaning mice 21 days old by intra cerebral route, it is not pathogenic intra peritoneal route for the adult mouse. If we consider the NATHANIEL A YOUNG classification it would represent a fifth subtype. Its antigenic relationship with Mucambo and Pixuna are too far to allow classifying them in subtypes III et IV.

The protocol of virus identification showed one part that the strain isolated by Charles SERIE CA Ar 508 was different from Pixuna and of course, from Mucambo and Tonate and on the other part that two other strains CA Ar 4389 and CA Ar 19007 isolated from Culex portesi captured at Paramana station and Gallion were similar to it. We therefore have, in the same focus two different virus but of the same vector Culex portesi; their ecology seems then close and equally near that of Mucambo. This proves that the viruses of this group do not mutually exclude.

TABLEAU XV

KINETIC HEMAGGLUTINATION-INHIBITION TEST

STRAINS CA An 410 d (Tonate), CA Ar 508 a, CA Ar 19007, MUCAMBO, PIXUNA, CA H 73-379

ANTIGENS		IMMUNES ASCITIC FLUIDS															
		CA An 410 d			508 a			19007			Mucambo			Pixuna			73-379		
		30'	120'	20 H	30'	120'	20 H	30'	120'	20 H	30'	120'	20 H	30'	120'	20 H	30'	120'	20 H
CA An 410 d	4 U.	320	>640	>640	<10	20	80	160	320	>640	40	80	160	40	40	80	>640	>640	>640
	8 U.	320	320	320	<10	<10	20	160	160	>640	40	40	160	20	20	40	320	320	>640
	16 U.	80	160	320	<10	<10	20	80	160	160	40	40	80	20	20	20	160	320	>640
CA Ar 508 a	4 U.	10	20	160	40	40	160	640	640	640	10	20	20	10	10	20	20	20	160
	8 U.	10	20	80	10	20	80	320	320	640	10	10	20	<10	<10	20	20	20	80
	16 U.	10	10	40	10	20	40	160	160	320	10	10	20	<10	<10	20	10	10	40
CA Ar 19007	4 U.	<10	<10	<10	20	40	160	320	1280	2560	10	20	20	<10	20	40	40	40	160
	8 U.	<10	<10	<10	20	40	160	320	640	2560	<10	10	10	10	20	40	40	40	160
	16 U.	<10	<10	<10	20	40	80	320	640	1280	<10	10	10	<10	20	20	40	40	80
Mucambo	4 U.	40	160	320	<10	20	80	160	320	2560	320	320	1280	80	80	320	160	160	1280
	8 U.	40	40	160	<10	20	40	160	160	640	320	320	1280	40	80	160	80	80	320
	16 U.	20	20	80	<10	<10	20	160	160	320	160	160	640	20	40	80	80	80	160
Pixuna	4 U.	20	40	40	10	40	40	640	640	640	10	20	20	1280	1280	1280	160	160	320
	8.U.	20	40	40	<10	10	20	160	320	640	10	20	20	640	640	640	80	80	160
	16 U.	20	40	40	<10	10	20	160	320	320	<10	10	20	640	640	640	80	80	160
CA H 73-379	4 U.	640	640	1280	10	10	20	320	320	640	80	80	320	80	40	160	1280	2560	10240
	8 U.	320	640	640	<10	<10	20	160	160	640	20	40	160	20	10	40	640	1280	10240
	16 U.	160	640	320	<10	<10	20	80	80	320	20	20	40	10	10	40	640	320	1280

The Mucambo isolation had been effected by SHOPE in the forest near Belem (Brazil) and that of Pixuna 94 kilometers in the south of Belem from Anopheles and from Trichoprosopon their coexistence was but an appearance.

As for Trinidad, if the strain "Trinidad Donkey" was isolated in 1943, they did not know if in this time it coexisted with Mucambo.

CA Ar 508 is easily separated from three other virus of the group through the simple complement fixation test. Moreover, we have compared the four strains of the VEE complex CA An 410 d(Tonate), CA Ar 508, Mucambo and Pixuna with kinetic hemagglutination inhibition test. We have added two strains belonging to the same complex and identified in complement fixation test, one CA Ar 19007 as similar to CA Ar 508 and another CA H 73-379 as similar to CA An 410 d(Tonate).

The mouse ascitic fluids used in this reaction are obtained after 3 injections of virus (Table XV).

This test confirms that the four strains CA An 410d, 508a, Mucambo and Pixuna are particularly different with the shortest time of incubation. The identification of CA H 73-379 with CA An 410d is confirmed in both way, immunserums with homologous and heterologous antigens and antigens with homologous and heterologous immunserums.

For CA Ar 19007 however the identification with CA Ar 508 is particularly manifest in the way antigen with the different immunserums, on the other hand the immunserum 19007 is less specific.

Meanwhile the complement fixation test permits to recognize CA Ar 19007 as similar to CA Ar 508 and different from CA An 410 d, Mucambo and Pixuna(Table XIII).

It seems well that this last reaction permit to separate the antigenic groups inside the VEE complex and make manifest two new ones whose prototypes strains are CA An 410 d(Tonate) and CA Ar 508.

The virus CA Ar 19007 similar to CA Ar 508 would allow, thanks to the kinetic inhibition hemagglutination reaction in the way, serum with the different antigens to serve as bond among the prototypes of the complex.

The Tonate virus is pathogenic for man, the isolation proceeding from acute serum of a patient, with serological conversion, proves it. The convalescent serum remarkably specific permits to confirm the identification of the virus strain from acute serum with Tonate and verify that they are both different of the three others VEE subtypes.

The presence of plasmodium falciparum in the blood of the patient could confirm the favoring part of the malaria in the isolation of strains of arbovirus.

The serological investigations realized in different points of the

department prove that the Tonate virus circulate in certain numbers of ecological nooks of the coastal zone.

It is often found in Regina-Kaw area, on the Approuague river where an investigation effected in some children shows that 35 % have been attacked. This virus can also be found in Cayenne as well as in its suburbs. In Cayenne on 286 serums it was found 11 % of positive. In Remire and Matoury small villages situated about 10 kilometers east of Cayenne and in the South the percentage are respectively 15 and 24 %.

In Sinnamary however no antibody carrier was found. This seems connected with the ecological conditions of this small village situated in the middle of a swamp zone where the preferential vector of Tonate, Culex portesi , is virtually absent.

Table IX indicate the antibody carriers for the three virus Mucambo , Pixuna and CA An 410d (Tonate) and emphasizes the exclusive circulation of the later. In fact we never find antibody isolated for Mucambo or Pixuna, the titer of hemagglutination inhibition test is always higher for Tonate than for the others and in complement fixation test often the antibodies are only detected with the Tonate antigen . We can only find a positivity for Mucambo and Pixuna, at minimum titer, at the unique case where the titer for Tonate is higher reaching 1/16 or 1/32. Mucambo does not seem then to produce natural attack of man and, for the time being, to have disappeared.

The preferential vector of CA An 410 d is as for Mucambo, Culex portesi it is the same for CA Ar 508, the two isolations of which came from the same vector. The biology and the trophic preferences of these mosquitoes are known. A work executed in Surinam by DE KRUIJF(12) could demonstrate that the vector is attracted preferencially by the rodents, man and birds. This mosquito if it gets its meals from man and birds, can without doubt transmit the virus from the bird to man or to the rodents. It is certain that Culex portesi which plays an important part as an arbovirus vector will form the subject of deep studies at the "Institut Pasteur" in Guyane.

However other vectors can interfere in particular in the suburbs of Cayenne, Wyeomyia melanocephala and Wyeomyia occulta. One unique isolation was made from Culex zeteki. On the other hand, on the coastal zone where the isolation from man was realized, Tonate was found in Culex spissipes and Mansonia titillans.

But this leads us to the most important part of this study. The isolation of the prototype strain was realized from a bird. We did not have this strain before in the laboratory, further more this strain was reisolated from the same pool of organs kept at low temperature. The first isolation then seems authentic and permit to think that the birds can disseminate the virus. The recognition of the existence of stable antigenic differences among the virus of VEE group and its characters of discontinuity seemed to be in favour of local evolution. As for the strain

antigenically near and geographically far the transmission could only be explained through the interference of birds. The isolation of the prototype strain of Tonate confirm it.

Psarocolius decumanus is a very common bird in the coastal area, it lives and nests in group. The geographically distribution is located between Panama of one part, the south-east of Brazil, the north-west of Argentine and Bolivia on the other part.

The serological investigations realized in rare birds whose size allowed a serum sampling showed that they did not have antibodies detected in hemagglutination inhibition test.

This does not apply to the rodents. On 39 rodents and marsupials recently captured 19, that is, 50 % presenting antibodies for one of the three virus of VEE group utilized,but the aspect of reaction is much more diversified than in man. It fact, if we have never found antibodies Mucambo isolated we have not either found pre-eminence of Tonate virus. Certain reactions are positive for Pixuna isolated(8) other for Tonate isolated(3) for Pixuna and Tonate(4) finally in four animals the antibodies are present for the three virus. These reactions seem to confirm the absence of Mucambo virus. It would have been interesting however that we introduce CA Ar 508 in the series of antigens, what we do now. We are related on Table XVI the Tonate isolations realized each month since the evidence of the prototype strain up to February 1975.

TABLE XVI

ISOLATIONS BY MONTH OF THE STRAIN CA AN 410d(TONATE) FROM JANUARY 1973 TO FEBRUARY 1975

	M O N T H											
Number of isolations	2	3	1	2	1	-	-	1	1	4	11	5

No isolation was realized during the last half of the rain season whereas on the contrary the maximum is situated during the dry season in the month of November. This seems to correspond to dissemination of Culex portesi which are affected by the abondant rains PANDAY(13) .

Concerning Una virus, the isolation of Psorophora ferox confirms the

role of this vector from where the prototype strain(14) is originated. The other isolations equally confirm the role of the Coquillettidia and of the Culex already enphasized by SERIE(10).

THE GROUP B VIRUSES.

The Dengue does not seem to circulate in Guyane now, and the last epidemic outbreak goes back to 1970. The serological investigations show that this virus has only circulated in Cayenne area and its suburbs. This correspond morever to the areas where the Aedes aegypti index kept high level in certain seasons. The control of this vector has always been obtained much more easily in the rural areas.

The survey programm had first called upon the military young men coming from Martinica and Guadaloupe for a more or less long staying. The taking of blood for testing was regularly effected. The greater part of these young men had already antibodies Dengue isolated upon their arrival and being vaccinated against Yellow fever we have seen to appear very large reactions of secondary type for the totality of the group B viruses used. Actually the survey program is based on the control upon arrival and leaving of military men coming from France in the department. No serologic conversion was noticed.

In the same group etiological role of Ilheus virus(16) in the febrile affections of benign evolution was confirmed(5)(7)through isolation of the virus from the blood of a patient.

It is interesting to note that the isolation was obtained through serum inoculation not only in the suckling mice but also in the mosquitoes Aedes aegypti by intra thoracic route. We could not find the vector but we could equally isolate this strain from the organs and blood of three birds captured : one on the border of the sea Piaya minuta, the other in the interior of the land at Maripasoula Momotus momota , the third Leistes militaris captured on the banks of a coastal river. This virus can then be found in the two large ecological areas : savannah of border of sea and huge forest.

THE GROUP C VIRUSES.

Murutucu was isolated from a patient showing a febrile syndrome of benign evolution analogous to the case observed in Brazil(5). The isolation was obtained after inoculating the serum by intra thoracic route to Aedes aegypti . The virus was isolated several times from Culex portesi but the role of Anopheles peryassui as vector did not seem to have been in evidence before.

OTHER GROUPS AND UNGROUPED VIRUSES.

We shall see very superficially the virus of group Guama because they cannot be differenciated by the techniques of complement fixation test and our program did not permit us to finish the identification of virus isolated.

However, it seems interesting to notice that a virus belonging to this group, CA An 265, was isolated and reisolated from the organs of a bird Galbula dea captured in Matoury near Cayenne. It concerns the first isolation from one of three virus Bimiti, Guama or Catu which had, so far always been found in the rodents.

Among the non identified virus and different of those we possess : CA An 1092 a, CA Ar 16102, CA Ar 16652; one CA Ar 16468 must be subject of more important investigation in man as well as in animals in order to try to know its cycle in the nature and its eventual pathogenic role. Isolated from the blood of a patient of whom we had little clinical information. He arrived at the Reanimation Service with a serious febrile hepatitis, we could not authentify the strain because the died. However if it was the only isolation realized that year and, in that time, this strain was not manipulated neither in the laboratory where the strains of reference are kept nor in the laboratory where the isolation are practised. These two laboratories are by the way far from each other. It seems then that it is authentic.

SUMMARY

The study of arbovirus in Guiana has begun by a serological investigation using a series of arbovirus chosen arbitrarily in fonction of the isolations already realized in this Department, then this serie is enriched of two virus : Ilheus and CA An 410 d (Tonate).

This permitted us to define a certain number of ecological foci where the virus circulation seemed important justifying that a surveillance program of vectors and virus reservoirs be established.

The study of the viruses of the Venezuelan Encephalitis group revealed particularly fruitful. In 1972 we could isolate the Mucambo virus from a batch of arthropodes of Lutzomyia genus caught on a coastal river near Cayenne, then a little later on March 1973 this same strain was found again four times in some organs of birds caught in the huge forest in Maripasoula. Since March 1973 this virus has not been found again. On January 1973 a virus CA An 410 d(Tonate) was isolated from organs of birds Psarocolius decumanus. The identification showed that it belonged the Venezuelan Encephalitis group but could easily be differenciated in complement fixation and neutralization test of Mucambo and Pixuna. It was then isolated many times from Culex portesi then from Wyeomyia melanocephala, Wyeomyia occulta, Culex spissipes, Culex zeteki and Mansonia titillans.

In 1973 CA An 410 d (Tonate) was isolated proceeding from a serum of a patient presenting a febrile syndrom with headaches. Antibodies were found with the complement fixation test in the convalescent serum. This same serum was particularly specific to separate CA An 410 d(Tonate) from Mucambo, Pixuna and from another virus of the same group, CA Ar 508. The serological investigations showed that the virus CA An 410 d(Tonate) was found again with a more or less significant frequence in man in Cayenne as well as in certain number of cities on the coastal area. The titer of antibodies for Tonate is always higher than that for Mucambo and Pixuna. These last ones have never been found isolated.

Some antibodies were equally found in the rodents serums and marsupials recently caught.

Two strains isolated from Culex portesi were identified as different from Mucambo, Pixuna and Tonate and similar to CA Ar 508 isolated in 1968 from Culex portesi by Charles SERIE.

The Tonate and CA Ar 508 isolations could be realized at the same time and in the same place from two batchs of Culex portesi proving thus that the viruses of this group do not exclude.

The differences in complement fixation test, seroneutralization and kinetic reaction of hemagglutination inhibition permit, it seems, to define the two prototypes Tonate and CA Ar 508 as two new sub-types of VEE group.

For the B group, there is no Dengue circulation actually, on the other hand, Ilheus could be isolated proceeding from febrile patient. This strain was found in the same time by inoculation to the suckling mice and to the mosquito Aedes aegypti by intra thoracic route. Ilheus was equally isolated from Piaya minuta and from Momotus momota.

In the C group Murutucu was found again in man after inoculation of the serum in the mosquito Aedes aegypti.

In the Guama group, 19 viruses were isolated from Culex portesi, Culex spissipes and from Coquillettidia venezuelensis. Onefact however is interesting, a strain belonging to this group was isolated from a bird Galbula dea.

Finally, four viruses different from each other and different of all the strains we have were isolated : from Pteroglossus aracari (CA An 1093 a) from Culex portesi (CA Ar 16102), from Aedes serratus and from Mansonia titillans(CA Ar 16468) and Anopheles peryassui (CA Ar 16652).

The strain CA Ar 16468 was equally isolated from the serum of a patient who died after his admission in the hospital. However, at the moment of the isolation this strain was not manipulated in any laboratory of the Institut.

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