Archives of Virology 83, 129—140 (1985)

Archives of Virology

The Isolation of Meaban Virus, a New *Flavivirus* from the Seabird Tick *Ornithodoros (Alectorobius) maritimus* in France

By

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With 4 Figures

Accepted July 25, 1984

Summary

Seven strains of a new *Flavivirus*, for which the name of Meaban virus is proposed, were isolated from the seabird tick *Ornithodoros (A.) maritimus* collected during 1981 and 1982 in nests of herring gulls *(Larus argentatus)* on islands of South Brittany, France. The new virus was compared serologically with 65 other flaviviruses including Tyuleniy virus and was found to be most closely related to, but different from Saumarez Reef virus, an agent previously isolated in Australia and Tasmania. Some general properties of Meaban virus are described and its antigenic relationships with other tick-borne flaviviruses associated with seabirds are discussed.

Introduction

Among some thirty-five arboviruses isolated from ticks associated with seabirds (3, 9) only five belong to the *Flavivirus* genus: Tyuleniy (TYU) virus isolated from *Ixodes (Ceratixodes) uriae* in U.S.S.R., Norway and Alaska (8, 17, 18, 21), West Nile (WN) virus from *Ornithodoros (Alectorobius) maritimus* (as *O. capensis*) in the Caspian sea (11), Saumarez Reef (SRE) virus from *Ixodes eudyptidis* and *O. (A.) capensis* in Australia (22), Russian Spring Summer Encephalitis (RSSE) virus from *I. uriae* in U.S.S.R. (2), and the CSIRO 122 strain ("Gadget's Gully virus"), a probable new *Flavivirus* from *I. uriae* in the Macquarie islands (15; St. George, pers. comm., 1983).

Both SRE and TYU viruses are thought to be potential pathogens for seabirds and man (18, 22). WN virus, a normally mosquito-borne virus, is responsible in human beings for a mild febrile illness, occasionally followed by a meningo-encephalitis, and is endemic or epidemic from South-Africa to South Europe and from the Mediterranean area to South-eastern India (1).

Multiple isolations in France from *O. (A.) maritimus* ticks of a new arbovirus belonging to the *Flavivirus* genus and closely related to, but different from SRE virus add to our knowledge of the geographical distribution and phylogenetic relationships of these agents.

The name of "Meaban virus" is proposed for this new agent according to the location of its first isolation. Brest/Ar/T707 strain was designated as the prototype strain.

Materials and Methods

Tick Collections

O. (A.) maritimus VERMEIL and MARGUET, 1967 (24) were collected in Southern Brittany, France, in 1981 and 1982, from different seabirds reserves. Meaban virus was isolated from specimens collected in two locations: 1. Meaban island, "Golfe du Morbihan" (47° 31 N — 2° 56 W), where 147 ticks (mostly engorged) were collected in nests of herring gulls (*Larus argentatus* Pont.) on July 3, 1981. Meaban seabird reserve was established in 1958. At that time, the breeding populations were largely Common Terns *(Sterna hirundo)*, Roseate Terns *(S. dougallii)* and Sandwich Terns *(Thalasseus sandvicensis)* and occasionally Arctic Terns *(S. paradisaea)*. During recent years terns were gradually displaced and finally entirely replaced by gulls. 2. Penfred (or "Penn Fret") island, Glenan Archipelago (48° 15 N — 3° 58 W), where 59 ticks were obtained on May 4, 1982, also from herring gulls' nests. In the past, terns of the species described above occupied many flat islands of the archipelago. They were also displaced by gulls in recent years.

Penfred island is located about 80 km from Meaban island.

Isolation Procedures and Virological Studies

Isolation procedures were conducted as previously described (6). Ticks were triturated in pools averaging 5—6 adults and 10—20 nymphs. The diluent was MEM with 2 per cent calf serum, antibiotics and amphotericin B. Triturated pools were centrifuged at 3000 rpm for 15 minutes at 4° C and then stored at —70° C. Pools were inoculated intracerebrally (i.c.) into 24—48 hours old suckling mice (s.m.).

When paralytic symptoms occurred, the strain was adapted to s.m. by serial i.c. passages using 10^{-2} suspension in the same diluent. Attempts to adapt further the s.m. isolates to tissue cultures were made using MRC 5, VERO, BHK 21 and primary monkey kidney cells propagated by standard methods.

When viruses were isolated, the strains were reisolated from the same material held at —70° C.

Virological studies of isolates were carried out in 21 day-old mice inoculated by the i.c. route. For titrations, end points were calculated by the method of REED and MUENCH (20). Tests included filtration through Millipore filters with 220 nm pores and effects on infectivity of diethylether, acidity and heating at 60° C for 1 hour.

Serological Studies of the Isolates

Antigens were prepared from infected s.m. brain by sucrose-acetone (7) or freon 113 extractions. Hemagglutination with 24 hour-old chick and goose erythrocytes was examined at pH 5.8 to 7.4 and at 4°, 20° and 37° C. Hemagglutination inhibition (HI) tests were performed following CLARKE and CASALS (7) using micromethods.

For complement fixation (CF) tests, antigens were box titrated against homologous and heterologous immune sera or ascitic fluids. Immune sera against the isolates were obtained in weanling mice by 2 intraperitoneal (i.p.) injections of 10 per cent live virus suspensions 10 days apart and collected 7 days after the last injection. Occasionally sera prepared by a single i.c. or i.p. injection were also used.

Ascitic fluids used at Y.A.R.U. were prepared by inoculating adult female mice with a 10 per cent infected s.m. brain suspension mixed with an equal volume of complete Freund's adjuvant. Mice were inoculated on days 0, 7, 14 and 18; on day 18, an additional injection of adjuvant and saline was administered. Ascitic fluids were tapped at weekly intervals thereafter.

For grouping the isolates, CF antigens were tested against a number of group or polyvalent immune ascitic fluids (IAF) supplied by the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland, U.S.A.

For typing the isolates, HI, CF and neutralization (NT) tests were performed using IAF or immune sera kindly supplied by the Yale Arbovirus Research Unit (Y.A.R.U., A. J. Main), the Rocky Mountains Laboratory (R.M.L., C. E. Yunker and C. M. Clifford) and the Queensland Institute of Medical Research (B. M. Gorman).

For NT tests we used the "constant serum – variable dilutions of virus" method with weanling mice inoculated by the i.c. route. Serum-virus mixtures were incubated for 1 hour at 37° C. Fresh guinea pig serum was used as accessory factor.

Human sera were tested by HI tests using Kaolin for the removal of non-specific inhibitors (7).

Pathological and Electron Microscopy (EM) Studies

Light microscopy of coronal sections was performed with s.m. brains infected with each of the isolates.

EM was carried out only for the Brest/Ar/T707 strain. Grids were examined and photographed with a Zeiss EM9 microscope at 60 kV.

Results

Seven viral strains, apparently identical when compared by cross HI tests (data not shown) were isolated from ticks collected in 1981 and 1982: five from Meaban island (Brest/ar/T707, T711, T712 and T715) and two from Penfred island (Brest/Ar/T901 and T905).

Minimal isolation rates (MIR) were identical (0.034) for the two sites: this probably reflects stable and ancient infections in these islands.

Table 1. *Brest/Ar/T707 sensitivity to a lipid solvent, acidic pH, and heat*

	Titre after exposure to		
Titre without exposure	Diethyl ether	pH 3.0	60° C (1 hour)
$10^{7.3}$/0.03 ml	$10^{1.5}$/0.03 ml	10^{2}/0.03 ml	$10^{4.2}$/0.03 ml

Table 2. *Results of HI tests performed against a limited number of flaviviruses*

Antigens (4—8 units)	Immune sera against Brest/Ar/T 707, obtained by		
	One i.c. injection of virus	Two i.p. injections	One i.p. injection
Brest/Ar/T 707	*1280*	*1280*	*80*
Wesselsbron	640	320	10
Tick-borne encephalitis, (Hypr)	20	20	0
West Nile (K 99)	640	640	40
Dengue type 2	20	80	0
Yellow fever, 17 D	160	320	10

Isolate Brest/Ar/T 707 was designed as the prototype strain. Subsequent studies were only carried out on the prototype strain.

Virological Properties

After inoculation of the suspensions, paralysis occurred in 100 per cent s.m. in 15 days and as early as the second i.c. passage the incubation period was reduced to 5 days. At its third i.c. passage, the virus was pathogenic for s.m. by both i.c. and i.p. routes and for weanling mice only by the i.c. route. It passed through a 220 nm membrane and was clearly diethyl-ether, acid and heat labile (Table 1). The strain did not adapt to the mammalian cells.

Reisolation succeeded 6 months after the original isolation.

Serological Properties

Hemagglutinin was detected at the second i.c. passage in s.m. brain with a pH range of 5.8 to 6.8 and an optimal titer of 1 : 4,096 at 4° C and pH 6.4.

In CF tests, a 1 : 40 dilution of this antigen gave negative reactions with A, Bunyamwera, California, Kemerovo, polyvalents 1, 4, 5, 10, Congo, Quaranfil, rabies and LCM IAF but a positive reaction with group B fluid (1 : 512).

In addition, Brest/Ar/T 707 immune sera reacted in HI tests with 5 flaviviruses currently handled in the Brest Virus Laboratory confirming the previous CF grouping (Table 2). These results also indicated that

Table 3. *Results of neutralisation tests comparing viruses isolated from ticks associated with seabirds*

Viruses	Immune ascitic fluid or serum against			
	T 707 (Brest)	SRE (YARU)	TYU (RML)	WN (RML)
T 707	*5.5*	6.6	5.2	3.2
SRE	1.9	*2.6*	0	—
TYU	0	0.7	*5.9*	—
WN	0	—	—	*4.6*

Table 4. *Complement-fixation and hemagglutination-inhibition tests comparing Brest/Ar/T 707 with other group B viruses*

	Brest/Ar/T 707			
	Antigen		Antibody	
	CF Ht/Ho[a]	HI Ht/Ho	CF Ht/Ho	HI Ht/Ho
Murray Valley encephalitis	16/64	10/160	256/4096	640/81920
Tyuleniy (FinV-724)	16/128	40/1280	256/4096	5120/81920
Sepik	128/>1024	160/>10240	256/4096	320/81920
CSIRO 122 (Gadget's Gully)	<8/64	<10/40	256/4096	160/81920
Saumarez Reef	128/256	80/160	128/4096	160/81920
Usutu	128/256	160/1280	128/4096	640/81920
Tyuleniy (TAR)	64/256	160/>10240	128/4096	1280/81920
Banzi	<8/32	<10/20	128/4096	640/81920
Ilheus	64/512	10/5120	128/4096	2560/81920
Wesselsbron	<8/64	<10/40	128/4096	1280/81920
Apoi	<8/128	10/160	128/4096	640/81920
Edge Hill	<8/128	10/80	128/4096	640/81920
TBE. RSSE	64/128	20/1280	64/4096	80/81920
Tyuleniy (LEIV 6c)	512/>1024	320/2560	64/4096	160/81920
Entebbe bat	<8/16	<10/40	64/4096	40960/81920
Israel Turkey encephalitis	64/256	320/640	64/4096	640/81920
Kadam	16/64	40/640	64/4096	1280/81920
Royal Farm	64/256	10/5120	64/4096	80/81920
Ntaya	<8/32	<10/160	64/4096	10240/81920
Stratford	<8/32	—	64/4096	—
Tembusu	<8/32	<10/160	64/4096	640/81920
West Nile	64/512	10/>10240	64/4096	640/81920
Langat	16/128	10/640	64/4096	320/81920
Dakar bat	<8/64	<10/10	64/4096	80/81920
Spondweni	<8/64	<10/40	64/4096	40/81920
Saboya	32/512	160/10240	64/4096	10240/81920
Dengue 3	16/512	10/80	64/4096	80/81920
Bussuquara	<8/256	<10/80	64/4096	640/81920
Uganda S	32/64	<10/80	32/4096	640/81920
Dengue 4	32/64	<10/40	32/4096	320/81920
Kyasanur Forest disease	64/256	10/160	32/4096	160/81920
Louping Ill.	64/512	40/640	32/4096	80/81920
Batu Cave	16/128	10/640	32/4096	1280/81920
Powassan	<8/64	<10/40	32/4096	80/81920
BeAn 3276000	32/512	10/—	32/4096	—
TBE, Hypr	32/1024	40/640	32/4096	160/81920
Kunjin	16/128	80/2560	16/4096	2560/81920
U.S. bat salivary gland	128/1024	80/5120	16/4096	320/81920
St. Louis encephalitis	128/1024	160/2560	16/4096	640/81920
Alfuy	16/256	20/160	16/4096	160/81920
Carey Island	16/256	40/—	16/4096	—

[a] Heterologous titre/homologous titre

Table 4 (continued)

	Brest/Ar/T 707			
	Antigen		Antibody	
	CF Ht/Ho[a]	HI Ht/Ho	CF Ht/Ho	HI Ht/Ho
Jugra	32/512	20/640	16/4096	<10/81920
Negishi	—	—	16/4096	40/81920
Cowbone ridge	16/8	10/320	16/4096	320/81920
Bukalasa bat	<8/64	10/40	8/4096	320/81920
Sikulu	—	—	8/4096	10/81920
Dengue 1	32/64	<10/80	<8/4096	160/81920
Bouboui	64/256	160/320	<8/4096	—
Kokobera	<8/32	<10/10	<8/4096	160/81920
Japanese encephalitis	<8/32	<10/20	<8/4096	320/81920
Dengue 2	8/64	<10/20	<8/4096	<10/81920
Phnom Penh	64/512	80/1280	<8/4096	320/81920
Rocio	32/512	20/—	<8/4096	—
Modoc	8/128	10/80	<8/4096	40/81920
Yellow fever	8/128	10/320	<8/4096	640/81920
Montana myotis leucoencephalitis	64/>1024	<10/—	<8/4096	—
Tamana	<8/128	<10/80	<8/4096	<10/91820
Zika	16/512	20/160	<8/4096	320/81920
Jutiapa	16/512	10/320	<8/4096	160/81920
Yokose	—	—	<8/4096	20/81920
Aroa	—	—	<8/4096	<10/81920
Koutango	—	—	<8/4096	—
Karshi	—	—	<8/4096	—
Omsk hemorrhagic fever	16/—	20/—	—	—
Bagaza	128/—	40/—	—	—
Polyvalent group B	32/—	40/10—640	—	—

Brest/Ar/T707 was not a strain of tick-borne encephalitis virus, an agent previously isolated in France (12) and closely related to RSSE virus isolated from *Ixodes uriae* ticks and common murres *(Uria aalge)* in the Barents Sea, U.S.S.R. (2).

Brest/Ar/T707 was also compared in NT tests with three other flaviviruses associated with seabirds; results showed the virus was different from WN, TYU and SRE viruses, although nearest to SRE (Table 3). In these tests, homologous SRE neutralization titre (2, 6 logs) was surprisingly low as compared with the substantial cross neutralization of Meaban virus by this antibody (6.6 logs); we have no explanation for this phenomenon. Both SRE and Meaban viruses were at their 4th passage in mice when used in NT tests.

Finally, Brest/Ar/T707 was sent to Y.A.R.U. where it was compared by HI and CF tests with all the available flaviviruses and variants in the

collection. These tests showed the strain was unique, differing from 65 other flaviviruses.

In CF test, the closest relationships were demonstrated with Murray Valley encephalitis, Sepik, Tyuleniy (Fin V — 724), CSIRO 122 and Saumarez Reef viruses (Table 4).

In HI tests, Brest/Ar/T707 appeared to be related to Entebbe Bat, N'taya, Soboya and more distantly to Tyuleniy (Fin V — 724). More distant relationships occured with CSIRO 122, Saumarez Reef and the original strain of Tyuleniy virus (Leiv 6c) (Table 4).

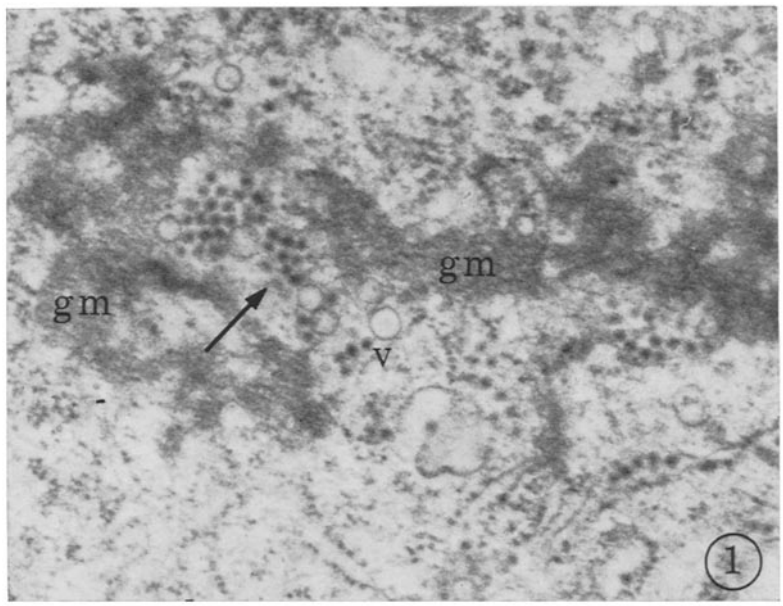

Fig. 1. Meaban virus. Infected s.m. brain. Intracellular virions (→) associated with vesicles *(v)* and granular matrices *(gm)* in the cytoplasm of an infected neuron (38,000×)

Light and Electron Microscopy

By light microscopy, s.m. brains infected by the different strains exhibited marked signs of acute meningo-encephalitis: meningeal infiltration by round cells, vasculitis and pericapillary cuffing and diffuse neuronal necrosis.

In EM, only Brest/Ar/T707 lesions were investigated. Meningo-encephalitis alterations were found again. Virus particles were seen in all the surveyed areas. Virions were located in the cytoplasm of injured neurons and in intercellular spaces. In the cytoplasm, particles were seen in dilated vesicles and in clusters associated with granular matrices (Fig. 1). Viral particles were typical for flaviviruses, exhibiting a dense core and a translucent envelope (Fig. 2). The mean diameter was 45 nm with extremes of

40 and 50 nm. As previously reported by MURPHY (19) no peculiar event concerning the maturation of virus particles was found. No core precursor, budding figure nor intermediate stage was identified.

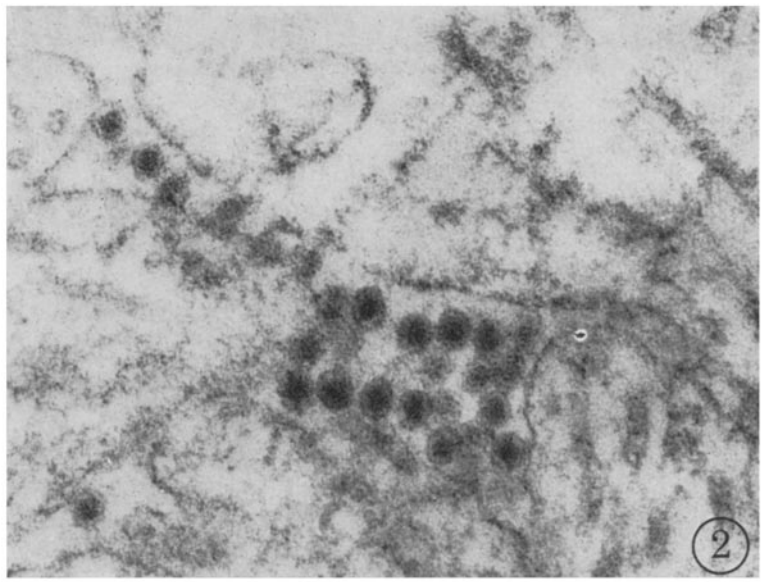

Fig. 2. Meaban virus. Infected sm brain. Typical extracellular virions exhibiting a dense core and a lucent envelope (100,000×)

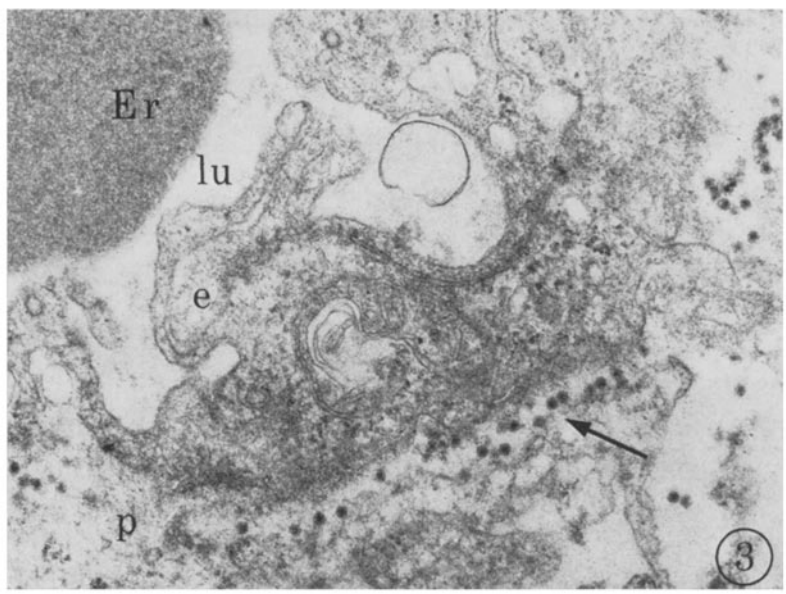

Fig. 3. Meaban virus. Injured capillary in infected s.m. brain. Virions are located in a single layer (→) between an endothelial *(e)* and a pericapillary glial cell *(p)*. Lumen of the capillary *(lu)*. Erythrocyte *(Er)*. (30,000×)

An interesting additional EM finding was the frequent accumulation of virions in a single layer between endothelial and pericapillary glial cells of an injured capillary (Fig. 3).

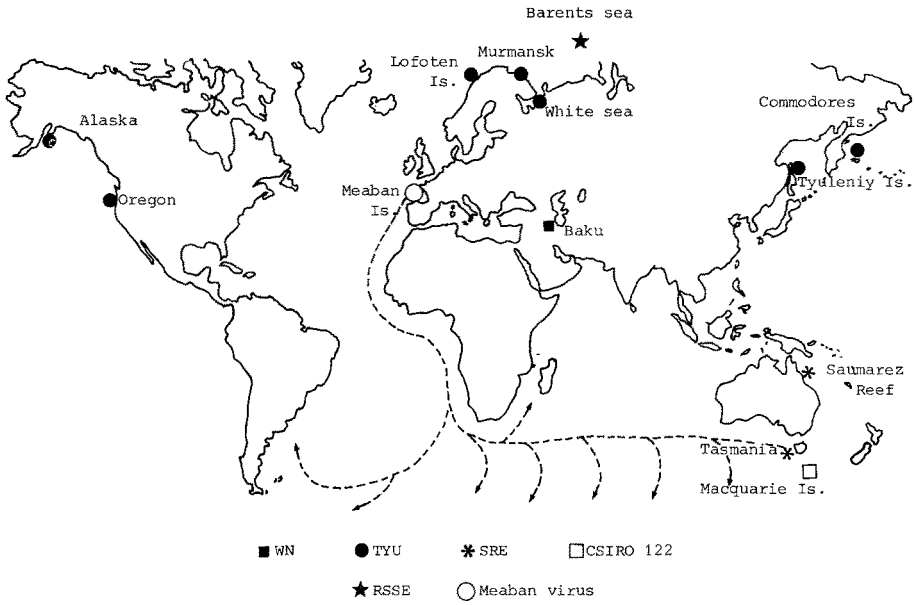

Fig. 4. Map of the world indicating the geographical distribution of tick-borne flaviviruses associated with seabirds and migratory routes of Arctic Terns from Brittany to Australia (– – →). (After K. CURRY-LINDAHL, 1980)

Discussion

Meaban virus is a new *Flavivirus* distinguished by both CF and HI tests performed against known viruses in this genus. It also differs from the five flaviviruses isolated from ticks associated with seabirds.

The morphology of Meaban virus particles in infected s.m. brain is quite typical of flaviviruses, the only peculiar feature being the frequent location of virions in a single layer between cells of capillaries (Fig. 3). This may have some implication in the pathogenesis of experimental meningoencephalitis induced in s.m. by this virus.

Meaban virus is the third *Flavivirus* isolated in France, the two others being West Nile virus from *Culex modestus* mosquitoes and human beings (13) and tick-borne encephalitis virus from *Ixodes ricinus* ticks (12). It is also the third tick-borne virus isolated from the tick species *O. (A.) maritimus*, after Soldado virus, a Hughes group virus of the genus *Nairovirus* from Europe (5, 14) and Morocco (4), and an *Orbivirus* of the Kemerovo group, Chenuda complex, from Morocco (4).

NT tests showed a closer relationship between Meaban virus and SRE virus than with TYU or WN viruses. TYU virus was isolated in the Northern hemisphere whereas SRE virus was isolated on Eastern coasts of Australia, at least 23,000 km from France.

Possible candidates for a past link between seabirds colonies in Brittany and Saumarez and Frederic Reefs would be terns, particularly Arctic Terns *(S. paradisaea)*, since they have long routes of migration from North Atlantic to South Africa, Antarctic and Tasmania (10, 23), but also common terns *(S. hirundo)*. Moreover, *O. (A.) maritimus* larvae were found on chicks of the common, roseate and sandwich terns breeding on islands of South Brittany (14).

However, our knowledge of the geographical distribution of tick-borne flaviviruses associated with seabirds is still far from complete, especially for the South Atlantic and Indian oceans. Furthermore, it is clear (see Table 4) that not all strains of TYU are identical, and more extensive testing of the various strains of TYU, SRE and Meaban viruses could well reveal different degrees of serological relationship.

In other respects, strong cross-reactions detected between Meaban and several mosquito-borne (Murray Valley encephalitis, Sepik, Usutu) and non-vector (Entebbe bat) flaviviruses were quite unexpected; they probably represent common epitopes shared between these viruses and possible another phylogenetic relationships.

Another important problem to be discussed is the possible pathogenicity of Meaban virus. We did not detect any Meaban virus HI antibody in 562 sera collected in human beings living in Brittany. However, the other tick-borne flaviviruses associated with seabirds are either demonstrated human pathogens (WN and RSSE) or suspected pathogens [TYU (16, 25) and SRE (22) viruses]. Precautions for any laboratory work with Meaban virus are essential.

Acknowledgements

This work was supported in part by grants from Ministère de l'Environnement, Paris (84-246) and from "Fondation Langlois", Rennes, France, and the U.S. Public Health Service, Grant AI 10984, Department of Defense Contracts N 000 14-78-C-0104 and DADA 17-73C-2170 and the World Health Organization.

The authors are indebted to Mrs. M. Odermatt and F. Le Goff for excellent technical assistance.

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Received June 11, 1984