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The isolation of arboviruses including a new flavivirus and a new Bunyavirus from Ixodes (Ceratixodes) uriae (Ixodoidea: Ixodidae) collected at Macquarie Island, Australia, 1975-1979. — Source link

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THE ISOLATION OF ARBOVIRUSES INCLUDING A NEW FLAVIVIRUS AND A NEW BUNYAVIRUS FROM *IXODES (CERATIXODES) URIAE* (IXODOIDEA: IXODIDAE) COLLECTED AT MACQUARIE ISLAND, AUSTRALIA, 1975–1979*

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Abstract. Pools of ticks, *Ixodes (Ceratixodes) uriae* collected between 1975 and 1979 at Macquarie Island, yielded 33 strains of at least 4 different viruses: Nugget virus (Kemerovo group), 1 strain; Taggert virus (Sakhalin group) 9 strains; a previously undescribed flavivirus, related to Central European Tickborne encephalitis virus, for which the name "Gadgets Gully" is proposed, 9 strains; a virus serologically related to the Uukuniemi serogroup, family Bunyaviridae,** for which the name "Precarious Point" is proposed, 10 strains. Three isolates were mixtures of Nugget and Gadgets Gully viruses; the remaining virus strain remains unidentified.

Macquarie Island, situated in the southern Pacific Ocean (latitude 54°30'S, longitude 159°E), is a national park with its only human inhabitants a small party of scientists. At sea level the air temperature varies between 0 and 10°C from winter to summer. Precipitation of 2,000 mm is spread throughout the year with some rain almost every day. Seabirds are abundant. Large populations of ticks *Ixodes (Ceratixodes) uriae* are associated with the penguin colonies. The viruses Nugget (Kemerovo Group) and Taggert (Sakhalin Group) were isolated by Doherty et al.[1] from ticks of this species collected on Macquarie Island. This report describes a continuation of that study.

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** Precarious Point virus was recently found to be a member of the Uukuniemi serogroup, Bunyaviridae at YARU.

MATERIALS AND METHODS

Macquarie Island (Fig. 1) is 30 km long by 5 km at its widest and has rocky, narrow foreshores, fringing cliffs on the east coast, and many pebbly beaches between headlands. The west coast has a narrow coastal plain covered with a dense mass of matted vegetation. There is a central plateau of 200–420 meters. It is on the beach areas and slopes immediately inland that the penguin colonies are found. All of the collections of the tick *I. (C.) uriae* were made from under rocks and debris or in tussock grass (*Poa foliosa*) in the vicinity of these colonies (Table 1). No collections were made from plateau or cliff areas where other seabirds nest. Ten widely spaced sites near the seashore were sampled. The species of penguin[2] inhabiting rookeries on Macquarie

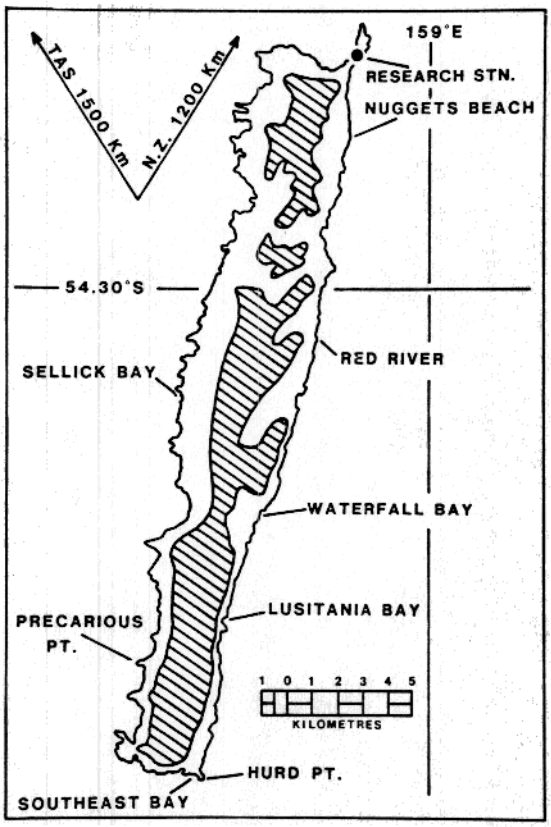

FIGURE 1. Map of Macquarie Island, showing the distribution of sites from which ticks were collected. The shaded area shows the part of the island 200 meters or more in height.

Island were Royal Penguins (*Eudyptes chrysolophus schlegeli*), King Penguins (*Aptenodytes patagonica*), and Rockhopper Penguins (*Eudyptes chrysocome*). However, the ticks were found almost entirely in the areas associated with Royal Penguins. The ticks collected, engorged or unengorged, were held for periods of 1 to 6 months at ambient temperature before being sent alive or in liquid nitrogen for storage in vaporous liquid nitrogen until processing for virus isolation.

The unengorged ticks were individually identified and separated into pools of 5 to 10 adults or up to 50 nymphs or larvae hatched from eggs. The preparation of the ticks for virus isolation, the intracerebral inoculation of mice, preparation of complement fixation (CF) antigen from positive mice, and serological techniques were as described by Doherty et al.[1] In addition, approximately 75% of the prepared pools were inoculated into baby hamster kidney monolayers (BHK21).[3] The cells were grown in Hanks' balanced salt solution with 0.5% lactalbumin 0.01% Difco yeast extract, 10% fetal calf serum, and 1,000 units each of sodium penicillin and streptomycin sulphate per ml. The medium was removed the day after inoculation with tick material and the cell sheet was washed to remove visible fragments of ticks. The medium was replaced with the growth medium in which the fetal calf serum content was reduced to 4%, and 50 units/ml of Nystatin were added. The cell sheets were observed 3 times a week for cytopathology. If cytopathology was noticed, the cells were passaged to further BHK21 monolayers. All monolayers without cytopathology were passaged twice at weekly intervals before being discarded as negative. Any virus isolated in cell cultures was adapted by intracerebral injection of mice for preparation of mouse brain antigens for identification.

Antigens and antisera (prepared in mice given 6 weekly intraperitoneal inoculations of infectious mouse brain against CSIRO 122 and MI 19334) were used in a range of tests against the Australian viruses or antisera held at the Queensland Institute of Medical Research. The serological techniques used at Yale Arbovirus Research Unit (YARU) were as described by Casals.[4]

RESULTS

A total of 123 adult ticks, 1,450 nymphs and approximately 500 larvae were received for processing for virus isolation. Delays in transshipment resulted in the death of many ticks en route or their overgrowth with fungus. Thirty-three strains of virus, as shown in Table 1, were isolated from 201 pools. The location of the 8 sites from which the infected ticks originated is illustrated in Figure 1.

Where ticks were inoculated into mice and cell cultures in parallel, strains were isolated in both systems in all 19 instances. Of the remaining 14 strains, 4 were isolated in mice and 10 in cell cultures but parallel isolation was not attempted. Complement fixation tests segregated the 33 strains into 6 groups, 2 of which were typified by the viruses Nugget and Taggert.[1] Two groups were antigenically unrelated types from which CSIRO 122 and MI 19334 were selected as prototype strains. Three isolates were mixtures of strains of MI 19334 and Nugget, and 1 strain

TABLE 1

Origin and identity of strains of viruses isolated from Ixodes *(C.)* uriae *collected from Royal Penguin rookeries on Macquarie Island*

Isolate number CSIRO	Stage of tick	Site and year of collection		Isolation system: Mice	Isolation system: Cells	Identity and type strain
90		Hurd Pt	1975			
122	Nymphal	Precarious Pt	1976	+	+	
127, 128, 135	Larval	Precarious Pt	1976	+	+	
129	Adult	Precarious Pt	1976	+	+	
146	Nymphal	Nuggets Beach	1977	+	+	
273, 274	Nymphal	Precarious Pt	1979	*	+	
MI 19334	Adult	Nuggets Beach	1975	+	*	"Precarious Pt"
120, 145	Adult	Lusitania Bay	1975	+	+	(MI 19334)
123	Nymphal	Lusitania Bay	1975	+	+	
124, 125	Nymphal	Precarious Pt	1976	+	+	
126, 142	Nymphal	Precarious Pt	1976	+	+	
136, 144	Adult	Hurd Pt	1977	+	+	
89	Adult	Red River	1976	*	+	Taggert
91	Adult	Sellick Bay	1976	*	+	(MI 14850)
121, 130	Adult	Hurd Pt	1977	+	+	
137, 138	Adult	Hurd Pt	1977	+	+	
267	Adult	Waterfall Bay	1979	*	+	
141, 143	Adult	Waterfall Bay	1979	*	+	
140	Adult	Nuggets Beach	1977	+	*	Nugget (MI 14847)
268, 269, 270	Adult	Southeast Bay	1979		+	Mixture Nugget and "Precarious Pt"
92	Adult	Sellick Bay	1976	+		Unidentified

* Isolation in the system not attempted.

remains unidentified. The following characteristics are reported for the 2 prototypes.

Gadgets Gully virus (CSIRO 122)

Initially, tests were carried out with CSIRO 122 and 127 in parallel. However, once the 2 proved to be identical then only CSIRO 122 was used in further tests and was sent to YARU.

Antigens from both CSIRO 122 and 127 viruses had a haemagglutinin at a pH above 6.4 and which was optimal at 6.8. In each case the hemagglutinin was inhibited by antisera to the Australian flaviviruses Saumarez Reef (CSIRO 4) and Murray Valley encephalitis (MRM 66) but not to the alphaviruses Sindbis (MRM 39) and Ross River (T48) viruses used in the initial screen. Both antigens were negative in a complement fixation test with antisera prepared against 2 strains of Saumarez Reef, Murray Valley encephalitis, West Nile, Kokobera, Kunjin, Edge Hill, Alfuy, Sepik, St. Louis encephalitis and dengue (types 1 to 4) viruses with the only positive finding being a low titer reaction to West Nile virus. A neutralization test in mice showed that CSIRO 122 was clearly distinct from Suamarez Reef virus, the only tick-borne flavivirus previously isolated in Australia (Table 2).

CSIRO 122 was confirmed as a flavivirus at YARU by CF and indirect immunofluorescence tests, being more closely related antigenically to the tick-borne than to other flaviviruses. It was, however, easily distinguishable from the available strains. The results of some of the CF tests are shown in Table 3, and the neutralization tests comparing CSIRO 122 with Central European tick-borne encephalitis virus (CETBE) are shown in Table 4. In addition, a hyperimmune mouse serum produced against CSIRO 122 gave negative reactions at a dilution of 1:8 with the following antigens: Banzi, dengue 2, dengue 4, Edge Hill, Kadam, Kyasanur Forest Disease, Kokobera, Kunjin, Murray Valley encephalitis, Saboya, Sepik, St. Louis encephalitis, Usutu and yellow fever viruses.

It is tentatively concluded that CSIRO 122 is

TABLE 2

Antigen comparison by HI, CF and neutralization tests of CSIRO 122 strain with Saumarez Reef virus (SRE)

Virus	Mouse ascitic fluid	
	SRE	CSIRO 122
Haemagglutination inhibiton		
SRE	≥5,120	640
CSIRO 122	160	320
Complement fixation*		
SRE	64/16	<8/<4
CSIRO 122	<8/<4	32/32
Neutralization†		
SRE	>3.6	1.8
CSIRO 122	0	1.6

* Reciprocal serum titer/reciprocal antigen titer.
† Neutralization index in infant mice inoculated intracerebrally.

a new flavivirus more closely related to some of the tick-borne complex than other flaviviruses.

The small amount of serology carried out on Macquarie Island birds showed that 2 of 30 Royal Penguin sera collected near Nuggets Beach (Fig. 1) and 1 of 6 skua sera (*Catharacta skua lonnbergi*) were reactive in a HI test with CSIRO 122 hemagglutinin. However, these same sera were also reactive against a haemagglutinin prepared from Murray Valley encephalitis virus. No neutralization tests were done.

Precarious Point virus (MI 19334)

The physical properties of strain MI 19334 are shown in Table 5. The haemagglutinin was active at a wide pH range but at a low titer and inhibition tests were not possible.

TABLE 4

Identification of strain CS-122—neutralization test by IC route in mice using antisera against CSIRO 122 and Central European tick-borne encephalitis (CETBE) viruses

Serum	Virus			
	CSIRO 122		CETBE	
	$ICLD_{50}$	Log NI*	$ICLD_{50}$	Log NI*

An homologous CF test was easily obtained with MI 19334 and after failing to find a reaction with any antisera to Australian arbovirus antigens and antisera the strain was sent to YARU.

At YARU, mouse immune serum or ascitic fluid for MI 19334 with homologous titers, 1:128–1:256, were tested in dilutions beginning at 1:4 or 1:8 against a large number of arbovirus antigens. Tests with the antigen prepared from MI 19334 were carried out against a wide range of grouping and specific sera, including mouse ascitic fluids prepared against 87 Bunyaviridae (Table 6). As the results of both these sets of tests were consistently negative it is concluded that MI-19334 is an agent distinct from and unrelated to any of the recognized arboviruses, and is presumably a new agent.

MI 19334 was characterized as a bunyavirus-like virus by electron microscopy. Thus it is possibly a new bunyavirus. However, a serological relationship has not been demonstrated with the known bunyaviruses. The identifying numbers and origin of the other 9 strains which were found

TABLE 3

A comparison of CSIRO 122 virus with a range of tick-borne flaviviruses by complement fixation

Antigen	Serum					
	CSIRO 122	CETBE	TYU	LAN	ZIKA	WN
			32+/32+			
	16/32+			32+/32+		
	8/16					
	8/4				32+/32+	
						32+/32+
	8/4					

* Reciprocal of serum titer/reciprocal of antigen titer.
** Central European tick-borne encephalitis.

TABLE 5
Characteristics of MI 19334 isolated from Ixodes *(C.) uriae*

Property	MI 19334
Titers of virus pool (suspension of third passage infected mouse brain) by:	
Intracerebral inoculation of infant mice*	8.3
Intraperitoneal inoculation of infant mice*	8.1
Intracerebral inoculation of weaned mice†	negative
Plaque assay on PS-EK cells‡	8.4
Haemagglutination of goose erythrocytes at pH§‖	6.0–6.6
Complement-fixation§¶	128/64
Sensitivity to sodium deoxycholate (1/1,000)**	6.1/2.0
Sensitivity to 50% diethyl ether**	≥7.5, <2.0
Titer ($log_{10}LD_{50}$/0.015 ml) after filtration through membrane filter of average pore diameter:	
450 nm	6.7
300 nm	6.7
220 nm	6.7
100 nm	3.0
No reduction of titer with IUDR. Persistence but not multiplication in inoculated *Aedes aegypti.*	

* $Log_{10}LD_{50}$ per ml.
† No evidence of paralysis at $10^{3.8}$ infant mouse LD_{50}.
‡ Log_{10} plaque-forming units per ml.
§ Sucrose-acetone extracts of infected mouse brain as antigen.
‖ Antigen diluted 1/10 and tested over pH range 6.0 to 7.6 at 37°C.
¶ Reciprocal serum titer/reciprocal antigen titer for homologous system.
** $Log_{10}LD_{50}$ per 0.015 ml of virus suspension incubated without (as control) or with sodium deoxycholate or ether (4°C for 18 hr).

to be identical to MI 19334 are listed in Table 1. No serological survey was carried out with this virus.

viruses

Nugget virus was represented by 1 strain (CSIRO 140) and 3 other strains of Nugget virus were mixed with MI 19334 in tick collections which originated from a split single collection of ticks from Southeast Bay. Taggert virus was isolated from 9 pools of ticks collected at 4 sites (Table 1).

The remaining strain CSIRO 92 was not identified, as an adequate homologous test system had not been developed at the time the work on this project was terminated.

DISCUSSION

The viruses isolated in this study originated from *I. (C.) uriae* ticks collected from sites occupied by colonies of Royal Penguins. The sites were well spaced around the shores of the island (Fig. 1). The isolation of Nugget virus at 1 site and Taggert virus at 5 sites additional to the single site which produced the original strains of Nugget and Taggert demonstrates that these viruses were not present only at the original site sampled on Macquarie Island.

The range and yield of viruses from ticks processed in this series is substantial but still does not reflect the full potential of the island. When the method of collection is considered over the time span covered, the number of ticks available for virus isolation was small. (Indirect methods of transport also were responsible for some losses.) However, tissue cultures were successfully used to isolate strains of Gadgets Gully, Precarious Point, Nugget and Taggert viruses.

The strains of virus were isolated from *I. (C.) uriae* collected on beaches on the eastern, western and southern coasts of the island. The other species of tick on Macquarie Island is *Ixodes kerguelenensis* (synonym *Ixodes pterodromae*).[5] It has been found only in the burrows of Dove Prions (*Pachyptila desolata*) in upland areas[6] and was not collected.

One of the new viruses, CSIRO 122, is a flavivirus clearly distinct from the other flavivi-

TABLE 6

Viruses in the family Bunyaviridae tested by complement fixation tests with MI 19334 mouse ascitic fluid

Serogroup	Virus
	Anopheles A, Lukuni, Tacaiuma
Anopheles B	Anopheles B
Bunyamwera	Germiston, Guaroa, Ilesha, Kairi, Sororoca, Tensaw, Wyeomyia
Bwamba	Bwamba
Group C	Apeu, Itaqui, Caraparu
California	California, Trivittatus, Melao
Capim	Capim, Guajara, Acara, Bushbush
Gamboa	Gamboa
Guama	Guama, Bertioga, Mirim, BeAn84381
Koongol	Koongol, Wongal
Nubututkab	Nubututkab
Oliflantsvlei	Oliflantsvlei, Bobia
Patois	Patois
Simbu	Simbu, Akabane, Manzanilla, Nola, Utinga, Thimiri, Oropouche, Sathuperi, Buttonwillow
Tete	Tete, Bahig, Tsuruse
Turlock	Turlock, Umbre, Yaba-1
Sand fly fever	Anhanga, Arumowot, BeAn100049, Bujaru, Chagres, Itaporanga, Karimabad, Pacui, Salehabad, Naples, Sicilian, Punta Toro, Candiru, SudAn 754-61
Crimean-Congo hemorrhagic fever	CCHF, Hazara
Dera Ghazi Khan	Dera Ghazi Khan, Abu Mina
Hughes	Hughes
Nairobi sheep disease	Dugbe
Qalyub	Qalyub, Bandia
Sakhalin	CloMor, Taggert
Uukuniemi	Uukuniemi, Grand Arbaud, EgAn 1825-61
Bakau	Bakau
Kaisodi	Silverwater, Lanjan
Maputta	Maputta
Yogue	Yogue
Unassigned	Belmont, Bhanja, Kowanyama, Lone Star, Tataguine, Witwatersrand, Keterah

ruses known to exist in the Australian, or subantarctic islands. However, there is a two-way relationship between CSIRO 122 and CETBE of the northern hemisphere. The tick, *I.* (*C.*) *uriae* of the southern hemisphere is also widely distributed in the subarctic (a.k.a. *Ixodes* (*Ceratixodes*) *putus*). CETBE virus has been associated with *Ixodes ricinus* and *Ixodes persulcatus* and not with *I.* (*C.*) *putus*. *I.* (*C.*) *putus* was associated with the type viruses of the Kemerovo and Sakhalin groups.[7-9] Nugget and Taggert viruses are members of these antigenic groups respectively[1] so that a common invertebrate host can be suggested as a possible link between the subarctic and subantarctic in the biology of these viruses, but so far no similar close link between CETBE and CSIRO 122 has been described.

The relationships found between *I.* (*C.*) *uriae* of the far north and south of the world, and the viruses they carry are intriguing. Doherty et al.[1] considered that the method of exchange of genetic material of viruses and ticks between the subarctic and subantarctic regions could not be explained by simple direct migration of particular species of birds between the land masses of these regions. However, in a review of tick-borne viruses of seabirds, Clifford[10] cites 2 species of seabirds which could fill such a role. Wilson's Storm Petrel (*Oceanites oceanicus*) breeds in the Antarctic and migrates to the Arctic. The reciprocal breeding and migration pattern is followed by the Arctic Tern (*Sterna paradisaea*).

Thus there may be a pathway whereby tick, and perhaps viral genetic material may be exchanged between the subarctic and subantarctic without it being established in the tropical zone in an unmodified form. The general distribution of *I.* (*C.*) *uriae* is illustrated by Clifford.[10] *I.* (*C.*) *uriae* has not been recorded between the tropics. The closest that *I.* (*C.*) *uriae* has been found to the tropics was a live female on a drowned Grey-headed Albatross (*Diomedea chrysostoma*), washed ashore on Fraser Island, Australia (25°S latitude).[11]

Until the ticks of the genus *Ixodes* that occur in the temperate and tropical regions are more thoroughly examined for Kemerovo, Sakhalin, CETBE viruses of the subarctic and Nugget, Taggert and CSIRO 122 viruses of the subantarctic, a discontinuity of distribution of such closely related viruses cannot be proven. There is scope for larger serological surveys to determine what species of seabirds are infected with these viruses to provide information on possible transfer pathways.

The bunyavirus MI 19334 is more difficult to categorize as no serological relationships with known viruses were demonstrated, and no se-

rological survey was carried out. Viruses of a number of bunyavirus serogroups have been isolated from ticks of the genus *Ixodes*, for instance of the Tete, CHF-Congo, Dera Ghazi Khan, Nairobi Sheep Disease, Sakhalin, Uukuniemi, Bhanja, Kaisodi and Thogoto serogroups.[12] The significance of MI 19334 as an infection or cause of disease of penguins or other subantarctic vertebrates must await future investigation.

This study has demonstrated that 2 viruses apparently new to science exist on Macquarie Island. The names which are proposed are associated with Macquarie Island history and are attached to geographical features near the points of collection. For the tick-borne flavivirus, CSIRO 122, we propose the name Gadgets Gully and for the bunyavirus, MI 19334, Precarious Point.

A total of 4 tick-borne arboviruses have now been found on Macquarie Island. In particular, the new tick-borne flavivirus related to CETBE warrants evaluation as a possible human as well as a bird pathogen. The total amount of work done on ticks and viruses from Macquarie Island is very small and clearly much more would be justified. Macquarie Island is small in physical size and isolated from large land masses so it may offer a most interesting ecosystem in which to study virus ecology in ticks and birds.

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