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FLAVIVIRUS ISOLATIONS FROM MOSQUITOES COLLECTED FROM WESTERN CAPE YORK PENINSULA, AUSTRALIA, 1999–2000

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ABSTRACT. After the 1st appearance of Japanese encephalitis virus (JE) on mainland Australia in 1998, a study was undertaken to investigate whether JE had become established in enzootic transmission cycles on western Cape York Peninsula. Adult mosquitoes were collected during the late wet season from Kowanyama and Pormpuraaw in April 1999, and Pormpuraaw and Barr's Yard in April 2000. Despite processing 269,270 mosquitoes for virus isolation, no isolates of JE were obtained. However, other flaviviruses comprising Murray Valley encephalitis virus, Kunjin virus, Alfuy virus, and Kokobera virus (KOK) were isolated. Isolates of the alphaviruses Ross River virus, Barmah Forest virus (BF), and Sindbis virus (SIN) also were obtained. The majority (88%) of isolates were from members of the *Culex sitiens* subgroup. Single isolates of KOK, BF, and SIN were obtained from *Ochlerotatus vigilax, Oc. normanensis,* and *Anopheles bancroftii,* respectively. The isolations of flaviviruses during the late wet season indicate that conditions were suitable for flavivirus activity in the area. No evidence was found to suggest that JE has become established in enzootic transmission cycles on western Cape York, although study sites and field trips were limited.

KEY WORDS Flavivirus, alphavirus, Murray Valley encephalitis virus, Kunjin virus, Queensland, Australia

INTRODUCTION

The 1st appearance of Japanese encephalitis virus (JE) on mainland Australia occurred during a widespread outbreak in northern Queensland and the Torres Strait in 1998 (Hanna et al. 1999). In addition to a confirmed human case on Badu Island in the Torres Strait, a 2nd human case was serologically confirmed in a fisherman working near the mouth of the Mitchell River, on western Cape York Peninsula. Seroconversions to JE were detected in sentinel pig herds on Badu Island and northern Cape York Peninsula (Hanna et al. 1999), and 43 isolates of JE were obtained from mosquitoes collected on Badu Island (Johansen et al. 2001). Furthermore, JE activity on western Cape York was confirmed when antibodies to JE were detected in young domestic pigs at Barr's Yard, a prison farm near the Mitchell River (Hanna et al. 1999). However, no other evidence of JE activity was detected in a widespread serosurvey of humans, and JE was not isolated from mosquitoes collected from northern and western Cape York Peninsula soon after the human case was recognized (van den Hurk et al. 2001a). This paper describes entomological and virological investigations undertaken on western Cape York Peninsula during the late wet season in 1999 and 2000, to examine indigenous flavivirus activity and the possibility that JE may have become established in enzootic transmission cycles in the region.

MATERIALS AND METHODS

Cape York Peninsula is located in far northern Queensland, Australia, and extends from 16°S to 11°S and 141°30′E to 146°E (Fig. 1). The peninsula is bordered by the Gulf of Carpentaria to the west and the Coral Sea to the east, and encompasses an area of approximately 137,200 km^2. The main rivers are the Mitchell and the Gilbert, which empty into the Gulf of Carpentaria. The climate is monsoonal. The average annual rainfall on western Cape York Peninsula is 1,200 mm (Biggs and Phillip 1995). More than 80% of the rain occurs during the wet season, between December and March. Temperatures are usually warm to hot, with maximum temperatures of >40°C recorded during summer. The study sites included the communities in the Mitchell River area of western Cape York Peninsula: Kowanyama (previously known as the Mitchell River Mission), Pormpuraaw (Edward River), and Barr's Yard. The population of western Cape York Peninsula in 1995 was approximately 1,450 (King 1995).

Mosquito collections were carried out between April 20–27, 1999, and April 3–7, 2000, when adult vector mosquitoes were likely to be most abundant. Adult mosquitoes were collected by using Centers for Disease Control (CDC)–type light traps baited with 1 kg of dry ice and octenol (release rate ca. 5 mg/h). Mosquitoes were identified to species on a refrigerated table and placed into pools of ≤100. The closely related *Culex annulirostris* Skuse, *Cx. palpalis* (Taylor), and *Cx. sitiens* Wiedemann were pooled as *Cx. sitiens* subgroup mosquitoes for analysis because of overlapping morphology, which makes identification of field-collected specimens difficult (Chapman et al. 2000). Pools were homogenized and inoculated onto 96-well monolayers of C6/36 cells. After 6 days of incubation the monolayers were fixed with acetone and assayed by tissue culture enzyme immunoassay (TC/EIA) (Broom et al. 1998) by using flavivirus group–reactive monoclonal antibody 4G2

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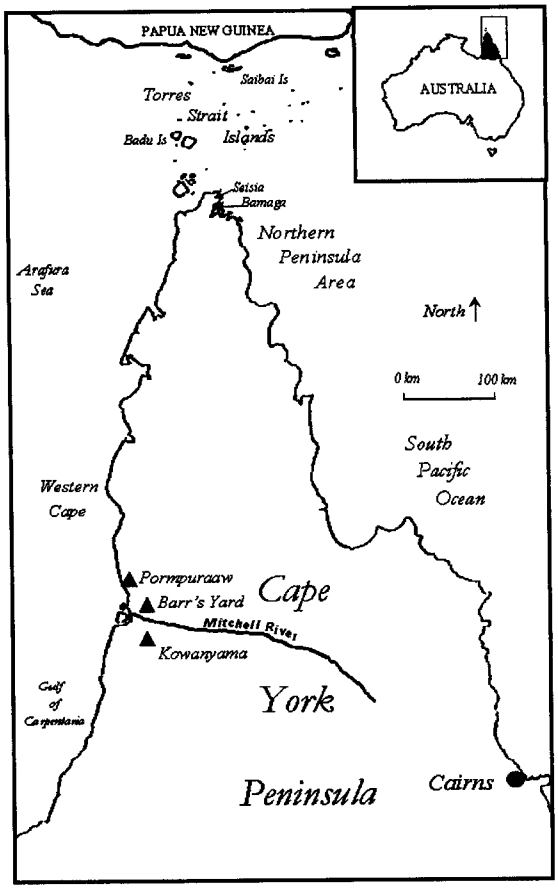

Fig. 1. Locations of adult mosquito collection sites on western Cape York Peninsula, 1999–2000.

(Henchal et al. 1982) for detection of flaviviruses. All isolates were confirmed by reisolation from the original homogenate and stocks were grown on porcine-stable equine kidney (PSEK) cells. Isolates were identified by inoculation of virus stocks onto 96-well monolayers of C6/36 cells, incubation for 6 days, and subsequent assay by TC/EIA with a panel of flavivirus-specific monoclonal antibodies (Johansen et al. 2000). Mosquito homogenates that caused cytopathic effect but were not identified as a flavivirus were subsequently assayed by TC/EIA with alphavirus-specific monoclonal antibodies (Johansen et al. 2000). Minimum infection rates (MIRs) were calculated by using a formula derived from Chiang and Reeves (1962).

RESULTS

A total of 269,270 adult mosquitoes collected from western Cape York Peninsula in 1999 and 2000, comprising 27 recognizable taxonomic units, were processed for virus isolation (Table 1). The most abundant species processed for virus isolation were members of the *Cx. sitiens* subgroup (59.8%), *Anopheles bancroftii* Giles (26.4%), *Ochlerotatus normanensis* (Taylor) (3.3%), *An. farauti* Laveran sensu lato (3.2%), *Cx. whitmorei* (Giles) (1.8%), and *Mansonia uniformis* (Theobald) (1.7%).

No isolates of JE were obtained from mosquitoes collected from western Cape York Peninsula in 1999 and 2000. However, other Japanese encephalitis serogroup viruses were isolated, including 17 Kunjin virus (KUN), 3 Murray Valley encephalitis virus (MVE), and 5 Alfuy virus (ALF) isolates (Table 2). One isolate of Kokobera virus (KOK) (a member of the Kokobera serogroup) was obtained, as were 4 isolates of Sindbis virus (SIN) and single isolates of Ross River virus (RR) and Barmah Forest virus (BF). Twenty-eight (87.5%) of the isolates were obtained from members of the *Cx. sitiens* subgroup. Kunjin virus was the only arbovirus obtained from mosquitoes collected in both years of the study period. Murray Valley encephalitis virus and ALF were only isolated from mosquitoes collected in 1999, from both Kowanyama and Pormpuraaw. The KOK isolate was obtained from a pool of *Ochlerotatus vigilax* (Skuse) collected at Pormpuraaw, and was the only flavivirus isolated from mosquitoes not belonging to the *Cx. sitiens* subgroup. Pools of *Oc. normanensis* yielded single isolates of BF and SIN and 1 isolate of SIN was obtained from *An. bancroftii.* Alphaviruses were only isolated from mosquitoes collected in April 2000. The overall infection rate of mosquitoes was generally low, with the highest MIR of 0.35 per 1,000 mosquitoes obtained with KUN isolated from *Cx. sitiens* subgroup mosquitoes collected from Kowanyama in April 1999.

DISCUSSION

No isolates of JE were obtained from mosquitoes collected on western Cape York Peninsula during the late wet season in 1999 and 2000, despite processing 269,270 mosquitoes for virus isolation. Laboratory studies have revealed that *Cx. sitiens* subgroup mosquitoes are vector competent for JE (van den Hurk et al. 2003), and it is likely that JE would have been detected had JE activity been present. The absence of JE isolations indicates either that the virus has not become established since initially detected in 1998 (Hanna et al. 1999), or that JE was not active in the study site at the time mosquitoes were collected. More sustained sampling over an extensive area may be required to elucidate this. Nonetheless, 4 other flaviviruses comprising MVE, KUN, ALF, and KOK were isolated, indicating that conditions were favorable for transmission of flaviviruses during the study period. Infection rates in mosquitoes were similar to those observed in mosquitoes collected from the Gulf Plains region of northern Queensland in 2000 (van den Hurk et al. 2002).

This is not the 1st time isolates of these viruses have been recorded from mosquitoes collected in

Table 1. Number of female mosquitoes processed for virus isolation from western Cape York, Australia, 1999–2000.

Species	No. processed (%)		
	1999	2000	Total
Aedeomyia catasticta Knab	0 (0.0)	19 (<0.1)	19 (<0.1)
Aedes lineatopennis (Ludlow)	66 (<0.1)	1,719 (1.5)	1,785 (0.7)
Anopheles amictus Edwards	318 (0.2)	54 (<0.1)	372 (0.1)
An. annulipes Walker sensu lato	104 (0.1)	0 (0.0)	104 (<0.1)
An. bancroftii Giles	45,590 (29.1)	25,494 (22.7)	71,084 (26.4)
An. farauti Laveran sensu lato	4,237 (2.7)	4,363 (3.9)	8,600 (3.2)
An. meraukensis Venhuis	0 (0.0)	5 (<0.1)	5 (<0.1)
Coquillettidia xanthogaster (Edwards)	86 (0.1)	43 (<0.1)	129 (<0.1)
Culex bitaeniorhynchus Giles	137 (0.1)	249 (0.2)	386 (0.1)
Cx. gelidus Theobald	1 (<0.1)	1 (<0.1)	2 (<0.1)
Cx. pullus Theobald	20 (<0.1)	2 (<0.1)	22 (<0.1)
Cx. sitiens subgroup[1]	93,813 (59.8)	67,126 (59.8)	160,939 (59.8)
Cx. vicinus (Taylor)[2]	2,233 (1.4)	0 (0.0)	2,233 (0.8)
Cx. whitmorei (Giles)	657 (0.4)	4,216 (3.8)	4,873 (1.8)
Mansonia septempuctata Theobald	101 (0.1)	0 (0.0)	101 (<0.1)
Ma. uniformis (Theobald)	4,462 (2.8)	183 (0.2)	4,645 (1.7)
Ochlerotatus kochi (Dönitz)[3]	1,595 (1.0)	432 (0.4)	2,047 (0.8)
Oc. littlechildi Taylor	0 (0.0)	3 (<0.1)	3 (<0.1)
Oc. normanensis (Taylor)	1,592 (1.0)	7,316 (6.5)	8,908 (3.3)
Oc. notoscriptus (Skuse)	34 (<0.1)	3 (<0.1)	37 (<0.1)
Oc. stoneorum Marks	0 (0.0)	1 (<0.1)	1 (<0.1)
Oc. palmarum Edwards	6 (<0.1)	0 (0.0)	6 (<0.1)
Oc. purpureus (Theobald)	2 (<0.1)	0 (0.0)	2 (<0.1)
Oc. rupestris Dobrotworsky	17 (<0.1)	85 (0.1)	102 (<0.1)
Oc. vigilax (Skuse)	1,651 (1.1)	994 (0.9)	2,645 (1.0)
Tripteroides magnesianus (Edwards)	2 (<0.1)	2 (<0.1)	4 (<0.1)
Unidentified *Culex* sp.[4]	159 (<0.1)	28 (<0.1)	184 (0.1)
Unidentified *Uranotaenia* sp.[5]	3 (<0.1)	0 (0.0)	3 (<0.1)
Verrallina carmenti Edwards	49 (<0.1)	0 (0.0)	49 (<0.1)
Total	156,932 (100.0)	112,338 (100.0)	269,270 (100.0)

[1] Comprised of the morphologically similar species *Cx. annulirostris, Cx. palpalis, Cx. sitiens*, and other undescribed species (Chapman et al. 2000).
[2] Identified as *Cx. vicinus*; recent findings indicate these mosquitoes were probably *Cx. gelidus* (Ritchie et al. 2001).
[3] Mosquitoes with profusely spotted wings, femora, and tibia in the *kochi* subgroup were classified as *Oc. kochi*
[4] Mosquitoes belonging to the genus *Culux*, but were unable to be identified to species.
[5] Mosquitoes belonging to the genus *Uranotaenia*, but were unable to be identified to species.

western Cape York Peninsula. Indeed, all 4 viruses were 1st isolated from mosquitoes collected during seminal studies of arbovirus ecology undertaken at Kowanyama in the 1960s (Doherty et al. 1963, 1968). However, other than the isolations of KOK from western Cape York in 1998 (van den Hurk et al. 2001a), there was a paucity of information about recent activity of flaviviruses on western Cape York Peninsula.

Isolations of KOK from mosquitoes collected in western Cape York Peninsula in 1998 (van den Hurk et al. 2001a) and 1999, and of KUN in 1999 and 2000, indicate that they may be enzootic in the region. Furthermore, a recent serological study revealed that 1.0% and 0.2% of residents in communities on western Cape York Peninsula possessed antibodies to KUN and KOK, respectively (Hanna et al. 1999), providing evidence that these viruses are potential threats to the health of people living in the region.

Murray Valley encephalitis virus was detected in mosquitoes from Kowanyama and Pormpuraaw in 1999, Normanton and Karumba in 2000 (van den Hurk et al. 2002), Mt. Isa in 2001, and Burketown in 2002 (A. Pyke, Queensland Health Scientific Services, Brisbane, Australia, personal communication). Although MVE and KUN activity in mosquitoes from western Cape York in 1999 and 2000 was not linked to clinical cases, a concurrent widespread outbreak of Murray Valley encephalitis occurred across western and central Australia during the 2000 wet season, resulting in 14 human cases (Broom et al. 2001b). In the following year, MVE activity was again widespread in sentinel chickens in Western Australia, the Northern Territory, and New South Wales (Broom et al. 2001a), and cases of Murray Valley encephalitis were reported in the Northern Territory (Brown and Krause 2001) and Mt. Isa in western Queensland (Hills 2001). The regular MVE activity suggests that this virus may be enzootic or is a regular threat in the larger Gulf Plains region and lower western Cape York Peninsula. The low infection rates of MVE in mosqui-

Table 2. Minimum infection rates of mosquitoes processed for virus isolation from western Cape York, Australia, 1999–2000.

Locality	Date	Species	No. processed for virus isolation	Percentage of no. processed from locality	No. pools processed for isolation	No. isolates (MIR)[1]					
						KUN	MVE	ALF	KOK	RR	BF	SIN
Kowanyama	April 20, 1999	*Culex sitiens* subgroup	24,370	76.3	250	1 (0.04)						
	April 21, 1999	*Culex sitiens* subgroup	18,328	67.1	187	2 (0.11)	1 (0.05)	1 (0.05)				
	April 22, 1999	*Culex sitiens* subgroup	8,814	53.4	87	3 (0.35)		2 (0.23)				
Pormpuraaw	April 23, 1999	*Culex sitiens* subgroup	12,302	52.8	126	3 (0.25)	2 (0.16)					
	April 25, 1999	*Culex sitiens* subgroup	25,442	52.4	252	4 (0.16)		2 (0.08)				
	April 25, 1999	*Ochlerotatus vigilax*	1,359	2.8	206				1 (0.74)			
	April 3, 2000	*Culex sitiens* subgroup	15,444	60.8	169	2 (0.13)						1 (0.06)
	April 5, 2000	*Culex sitiens* subgroup	28,752	61.7	290					1 (0.03)		1 (0.03)
	April 5, 2000	*Anopheles bancroftii*	13,377	28.7	137							1 (0.08)
Barr's Yard	April 4, 2000	*Culex sitiens* subgroup	22,930	56.8	233	2 (0.09)					1 (0.14)	1 (0.04)
		Ochlerotatus normanensis	7,298	18.1	75							

[1] MIR, minimum infection rate per 1,000 mosquitoes, following Chiang and Reeves (1962); KUN, Kunjin virus; MVE, Murray Valley encephalitis virus; ALF, Alfuy virus; KOK, Kokobera virus; RR, Ross River virus; BF, Barmah Forest virus; SIN, Sindbis virus.

toes during this study may also indicate enzootic transmission.

The majority of flavivirus isolates were from *Cx. sitiens* subgroup mosquitoes, providing further evidence for the role of these species in transmission of flaviviruses. *Culex annulirostris* is a confirmed vector of MVE and KUN (Kay et al. 1984), and the vector competence of Australian *Cx. annulirostris* and *Cx. sitiens* for a Torres Strait JE strain has recently been confirmed in the laboratory (van den Hurk et al. 2003). Other species of mosquitoes collected from western Cape York Peninsula that are confirmed or suspected vectors of JE include *Culex bitaeniorhynchus* Giles (Banerjee and Deshmukh 1987), *Cx. whitmorei* and *Ma. uniformis* (Peiris et al. 1992), and *Aedes lineatopennis* (Ludlow) (Vythilingam et al. 1997). *Mansonia uniformis* can reach pest proportions in Australia, and could potentially have a role in transmission of JE on Cape York Peninsula. In contrast, *Cx. bitaeniorhynchus* and *Cx. whitmorei* were not collected in large numbers, and may have only a minor role in JE transmission. However, the collection of *Culex gelidus* Theobald, a newly recognized mosquito in Australia (Ritchie et al. 2001), on western Cape York may have implications for flavivirus transmission. Although only a single confirmed *Cx. gelidus* was collected in the western Cape York, large numbers of *Culex vicinus* (Taylor) were collected that may have been misidentified *Cx. gelidus* (Ritchie et al. 2001). It is also possible that *Cx. gelidus* is not well established at the locations where adult mosquitoes were sampled during this study. *Culex gelidus* has been collected in large numbers in CDC-type traps in northern Queensland (Johansen et al. 2003), indicating they are readily attracted to the mosquito collection traps used in this study. Furthermore, JE was isolated from *Cx. gelidus* collected on Badu Island in 2000 (van den Hurk et al. 2001b), and is considered an important vector of JE in southeast Asia (Vaughn and Hoke 1992). If JE reappears on Cape York Peninsula, further collections of mosquitoes for virus isolation should be undertaken to examine the potential for virus establishment, and the subsequent implementation of control programs to protect residents from infection needs to be considered.

The isolations of the alphaviruses RR and SIN are not surprising. Sindbis virus is the arbovirus most commonly isolated from mosquitoes in Australia (Mackenzie et al. 1994), and Queensland has the highest incidence of RR disease in Australia (Harley et al. 2001). Barmah Forest virus is also a common cause of arboviral disease in Queensland (Hills and Sheridan 1997). However, disease caused by SIN infection is rare in Australia (Mackenzie et al. 1994).

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