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ALPHAVIRUS INFECTION IN MOSQUITOES AT THE ROSS RIVER RESERVOIR, NORTH QUEENSLAND, 1990–1993

B. H. KAY,[1] M. N. HEARNDEN,[1,2] N. M. M. OLIVEIRA,[1,3]
L. N. SELLNER[3,4] AND R. A. HALL[2,5]

ABSTRACT. This study addresses the potential problem of alphavirus infection associated with recreational use of the Ross River reservoir in north Queensland, Australia. From 1990 to 1993, 51,497 adult female mosquitoes were collected mainly by CO_2-supplemented light traps. Four localities within the reservoir were considered and compared with mosquitoes collected during 1991 from 2 public localities around Townsville City. Ten isolates of Ross River virus, one of Barmah Forest virus, and 2 of Sindbis virus were recovered from *Aedes normanensis, Anopheles amictus,* and *Culex annulirostris.* All isolates were collected during the wet seasons of 1991 and 1992 using an enzyme immunoassay and cross-checked using a polymerase chain reaction assay. Estimation of relative hazard was based on total mosquito abundance, prevalence of vector species, and on mosquito infection rates. Based on 1990–93 data, it was concluded that the Big Bay area of the Ross River dam, currently being considered as a primary recreational locality, was relatively safer than Antill Creek, Ross River, and Toonpan and presented no greater hazard than localities around urban Townsville, away from the reservoir. However, because of the changing ecology of the reservoir and lack of a full understanding of annual alphavirus activity, periodic surveillance is recommended.

INTRODUCTION

The Ross River dam (19°26′S, 146°45′E), on the western boundary of the twin cities of Townsville–Thuringowa was constructed as stage 1 (1973) and stage 2A (1987) to augment increasing water supply demands. Construction of the second stage was planned in 2 steps, A and B, by progressively adding to spillway height using concrete. After the stage 2A reservoir filled to its maximum spillway level of 38.2 m in 1989, creating a lake of 8,090 ha, public pressure increased with respect to diverse recreational uses. Stage 2B has yet to be constructed.

Further details of the reservoir have been given by Kay et al. (1990) and Barker-Hudson et al. (1993). From these studies, it became apparent that mosquitoes and alphaviruses, such as Ross River (RR) virus were common. Between 1983 and 1987 when studies on the stage 1 reservoir were done, up to 84% of sentinel chicken flocks situated at or near the reservoir seroconverted to alphaviruses each year, whereas this was a comparatively rare event for flaviviruses (Murray Valley encephalitis, Kunjin, Kokobera, Edge Hill, Alfuy). For RR virus, chickens are considered to be inefficient sentinels as they do not always develop an antibody response and if they do, it is often low titer and transitory (Kay et al. 1986). Alphavirus activity was not associated with any particular study site, 2 near the reservoir and 2 within residential areas. Furthermore, antibody seroconversion rates showed both seasonal and annual variation.

In view of the ecological changes and an in-principle decision of the Townsville–Thuringowa Water Supply Board to open the dam for limited public use, our task was to define risk of arbovirus infection, both in spatial and temporal terms. From 1990 to 1993, collections were recommenced to evaluate mosquito populations at the Ross River reservoir and to evaluate any changes that may have occurred between stage 1 and stage 2A (Hearnden and Kay 1995). Due to clearing of woodland in the vicinity of stage 2A boundaries, several tree hole/plant axil and shaded pool species were eradicated or became rare. Mean abundance of one of the major species, *Culex annulirostris* Skuse, did not exceed densities recorded during 1984 to 1985 for stage 1. In contrast, numbers of *Anopheles amictus* Edwards and *Aedes normanensis* Taylor increased 36- and 282-fold, respectively. This was due to the loss of marginal emergent vegetation and the creation of expansive temporary muddy pools, suitable for their breeding.

Numerous strains of RR and Barmah Forest (BF) viruses have been isolated from these 3 freshwater species (Russell 1995) as well as

[1] Queensland Institute of Medical Research, Royal Brisbane Hospital P. O., Brisbane, QLD 4029, Australia.
[2] Department of Zoology, James Cook University P. O., Townsville, QLD 4810, Australia.
[3] Department of Microbiology, University of Western Australia, QEII Medical Centre, Nedlands, WA 6009, Australia.
[4] Present address: Department of Pathology, Royal Perth Hospital, Perth, WA 6000, Australia.
[5] Present address: Department of Microbiology, University of Queensland, Brisbane, QLD 4072, Australia.

from *Aedes vigilax* (Skuse), which breeds in coastal salt marsh. Although *Cx. annulirostris* and *Ae. vigilax* are regarded as the major vectors along the eastern seaboard, RR virus is catholic in its association with mosquito species, as it has been isolated from 28 species from 6 genera (Russell 1995). To date, transmission following oral feeding has been demonstrated in 16 of 17 species tested (Kay and Aaskov 1988; Fanning et al. 1992; Kay, unpublished data).

Culex annulirostris, An. amictus, Ae. normanensis, and *Ae. vigilax* are all known as opportunistic mammalian feeders (Kay et al. 1979, Kay and Aaskov 1988) and feed readily on humans. Both *Cx. annulirostris* (O'Donnell et al. 1992) and *Ae. vigilax* (Marks 1969) are known to disperse widely and are, therefore, panmictic throughout the area covered by this study. This implies that the population age structures of these two species would be similar, although it could be expected that more nulliparous females would be collected from sites nearest major breeding sites, for example, around the lake margins. Less is known of *Ae. normanensis* and *An. amictus.*

On this basis, therefore, we felt justified in examining certain key parameters that would show greater variability in the sites under consideration: species prevalence, abundance, and infection rates as a basis for an estimation of relative hazard. This choice was also influenced by practical consideration of future periodic management requests for risk assessments prior to recreational events.

MATERIALS AND METHODS

Study area: Following recommendations for potential recreational usage by the Department of Local Government (1985), areas on the northern (Big Bay and quarry area) and northeastern sides (Antill Creek) of the reservoir were chosen for study. Mosquito infection rates (Chiang and Reeves 1962) were estimated and compared with those from Ross River, entering the lake from the southwest, Toonpan at the southeastern end away from the dam wall, and to a more limited extent, with 2 Townsville sites (Town Common, Townsville Residential).

Mosquito collections: A detailed description of the trapping methods and seasonal conditions was presented in Hearnden and Kay (1995). From February 1991 to June 1993, adult mosquitoes were collected mainly at Big Bay, Antill Creek, Toonpan, and Ross River around the reservoir by using 6 CO_2-supplemented encephalitis virus surveillance (EVS) traps (Rohe and Fall 1979) monthly at each locality. Traps were suspended 0.5–1 m above the ground and modified with a photoresistor to start them at dusk and turn them off at dawn. The design also incorporated a self-closing baffle to prevent the escape of mosquitoes from the trap bag.

Rainfall was characterized by a wet season (generally from December to March), which was typical during 1990–91 but much lower than usual during 1991–92 and 1992–93. The sites could be described as follows: Big Bay (northern shoreline) and Ross River (southwestern shoreline) were well vegetated with open *Eucalyptus* spp. woodland. Paperbark ti-trees, *Melaleuca* spp., were also present at Ross River. These trap localities remained relatively close to the water throughout the study. Antill Creek to the northeast and Toonpan to the southeast were extensively cleared of vegetation prior to flooding of stage 2A. Because of the shallowness of the lake, the shoreline receded quickly during the dry season exposing bare mudflats interdispersed with occasional dead Chinee apple bush, *Zizyphus mauritania.* By November these trap localities were sometimes 2 km away from the shoreline. During February and May 1991, further light trap collections were made at the Townsville Common, a coastal wetlands reserve influenced by tides. It lies on the northern margin of residential Townsville, where traps were also set.

Between May and September 1990, supplementary human bait collections were carried out for 1 h after sunset at Ross River reservoir localities (32 collections) and at the Town Common (2 collections). These were included for virus processing, but not for calculating mean catches.

Alphavirus detection: African green monkey kidney (Vero) and porcine squamous equine kidney (PSEK) cells were grown in M199 (GIBCO) medium supplemented with 10% fetal bovine serum (FBS) and 20 mM HEPES and incubated at 37°C. *Aedes albopictus* (C6/36) and baby hamster kidney (BHK) cells were grown in the same medium without HEPES, at 28°C and 37°C, respectively. Cell lines were incubated in a CO_2-enriched atmosphere.

Monoclonal antibodies (Mabs) specific for RR (K1503 3B2) and/or cross-reactive with BF, chikungunya, Getah, and Semliki Forest viruses (T48B10) were produced to T48 (prototype) and K1503 RR strains according to the methods of Galfre et al. (1977). Additional Mabs reacting with Sindbis and chikungunya (2G2) and Getah and Semliki Forest (T48 A8) viruses were also used in the screening process to positively identify BF and Sindbis viruses by elimination.

Processing of mosquitoes for virus isolation and identification: Mosquitoes were sorted to species in the laboratory on an electric chill ta-

ble at −10°C. For processing, pools of mosquitoes were placed in 5-ml tubes that contained 2.5 ml 2% FBS M199 (without HEPES) and 5-mm-diameter glass beads (5 beads were used for pool sizes of 30 or less mosquitoes and 8 beads were used for pool sizes greater than 30). Specimens were triturated for 3 min in pool numbers ranging from 1 to 134 using a Spex 8000 mixer mill. The ground mosquito homogenates were centrifuged for 15 min at 2,500 rpm at 4°C, and supernatants were harvested and aliquoted into sterile Eppendorf tubes and stored at −70°C until processing for virus isolation. All steps were carried out aseptically on ice.

Ground mosquito homogenates were inoculated onto confluent monolayers of C6/36 cells grown in 96-well trays (Falcon) and incubated for 4 days at 28°C in a humidified atmosphere. Fifty microliters of homogenate were inoculated per well in duplicate for pools of 29 or less mosquitoes and a 1/5 dilution of homogenate was made of pools with greater than 30 mosquitoes, as mosquito extracts from greater than this are toxic to C6/36 cell culture. Cells were observed for toxicity and positive supernatants were harvested.

To increase the sensitivity of the assay, 50 μl of the remaining supernatants were repassaged onto fresh monolayers of C6/36 cells and BHK cells. Cells were incubated for a further 4 days, and observed for toxicity on C6/36 cells and cytopathic effects (CPE) on BHK cells. A final passage was carried out from the C6/36 cells onto fresh monolayers of BHK and PSEK cells, which were incubated and observed as described above. If CPE was evident in any of the indicator cell lines, the supernatant was harvested for virus identification.

The parameters for the enzyme immunosorbent assay (EIA) of infected cell monolayers have been described previously (Oliveira et al. 1995). After each incubation period, all inoculated monolayers were fixed with 20% acetone in phosphate-buffered saline for 1 h at 4°C and dried overnight at 37°C. Nonspecific binding sites were then blocked with 100 μl of blocking buffer (0.2% w/v casein, 0.05 M Tris, 0.01 M EDTA, 0.15 M NaCl, pH 8.0) for 1 h at room temperature. Blocking buffer was then flicked out of the wells and RR virus-specific and alphavirus-crossreactive Mabs were used for the detection of viral antigens.

Mosquito homogenates were subjected to nested polymerase chain reaction (RT-PCR) (Sellner et al. 1994). Those chosen were the 10 positives by EIA plus 50 others at random. One hundred microliters of homogenate was mixed with 100 μl lysis buffer (8 M guanidine thiocyanate, 50 mM sodium citrate, 100 mM 2-mercaptoethanol, 1% sodium lauryl sulfate, 1 μg/ml yeast tRNA). Forty microliters of 2 M sodium acetate was then added, followed by 400 μl Tris buffered phenol, and 80 μl chloroform, mixing between each addition. The tubes were cooled on ice for 15 min, then centrifuged for 15 min at 13,000 × *g* at 4°C. The aqueous phase was removed to a fresh vessel, mixed with an equal volume of isopropanol, and cooled at −20°C for 1 h. Precipitated ribonucleic acid (RNA) was pelleted by centrifugation for 10 min at 4°C, and the RNA pellet resuspended in 20 μl water. Two microliters of this was then added directly to the tube for the nested RT-PCR reaction.

Each external primer RR 8717s and RR 9247 was 20 base pairs (bp) long and produced a 550-bp product. The second round reaction mixtures, containing 50 pmol each of internal primers RR 8958s and RR 9137 (both 14 bp long), produced a 193-bp product.

Assessment of relative hazard: Mean mosquito abundance expressed as number of mosquitoes per trap night, percentage of total catch formed by RR and BF virus-infected species, and their infection rates for each locality were jointly considered to form an estimate of relative hazard. Each parameter was expressed as a multiple of the lowest score, for example, for mean catch per night, the average catch of 247 at Townsville is 3.42 times that of 72.4 for Big Bay. For each locality, the comparative scores for each of the 3 factors, for example, Big Bay (1 × 1 × 2), were then multiplied together to give an overall score, indicating relative hazard. Because the nondetection of isolates from Townsville did not indicate that RR and BF viruses were absent, the last multiplier used was 1 rather than 0.

RESULTS

Mosquito collections: A total of 51,497 adult female mosquitoes comprising 24 taxa were pooled for virus detection according to locality around the reservoir and from the 2 Townsville sites (Table 1). Detailed analyses of their seasonal abundance (based on 50,430 mosquitoes from 509 light trap collections) and locality information for those collected around the reservoir have been given by Hearnden and Kay (1995). Peak periods of mosquito abundance were strongly seasonal, occurring in late to post-wet season months (March–May).

In summary, the mean number of mosquitoes per light trap night at each locality was 72.4, 72.6, 116.5, and 191 for Big Bay, Ross River, Antill Creek, and Toonpan, respectively. At Big Bay, 45.3% of catches were *An. annulipes s.l.*, whereas *Cx. annulirostris, An. amictus,* and *Ae.*

Table 1. Adult female mosquitoes pooled from collections from the Ross River reservoir and Townsville area for alphavirus testing, 1990–93.

Taxon	1990	1991		
	May–Sept.	Feb.	April–June	Dec.
Aedeomyia catasticta	7 (3)	6 (2)	31 (9)	48 (5)
Aedes alternans	37 (2)	1 (1)		
Aedes elchoensis		12 (1)	4 (1)	2 (2)
Aedes funereus	1 (1)			
Aedes kochi		10 (1)		
Aedes lineatopennis	18 (2)	67 (3)		
Aedes normanensis		5,464 (58)	3 (1)	89 (3)
Aedes notoscriptus	1 (1)	25 (3)	4 (1)	
Aedes vigilax	39 (6)	141 (1)	5,948 (58)	274 (9)
Aedes vittiger	159 (4)			
Anopheles amictus	374 (12)	169 (7)	2,130 (34)	1,829 (32)
Anopheles annulipes	651 (15)	135 (7)	1,007 (29)	1,546 (29)
Anopheles bancroftii	8 (4)		1 (1)	
Anopheles hilli	602 (8)	2 (1)		
Anopheles meraukensis	222 (9)	35 (4)	38 (9)	116 (7)
Coquillettidia crassipes		4 (2)	36 (3)	
Culex annulirostris	2,401 (31)	736 (12)	1,464 (20)	1,185 (16)
Culex bitaeniorhynchus	248 (5)	253 (6)	116 (6)	2 (1)
Culex pullus	4 (2)	37 (3)		
Culex quinquefasciatus	9 (3)			
Culex sitiens	2 (1)			
Culex squamosus				
Mansonia septempunctata	1 (1)			
Mansonia uniformis	355 (10)	53 (3)	117 (11)	50 (4)
Number collected (Number of pools processed)	5,139 (120)	7,150 (114)	10,449 (183)	5,141 (108)

normanensis comprised 21.4, 16, and 10.4%, respectively. At Ross River, Antill Creek, and Toonpan, *Ae. normanensis* comprised 46.2, 28.3, and 28.3%, respectively, whereas at the latter 2 localities, *An. amictus* comprised 43.2 and 55.2% of the total mosquitoes collected. At these reservoir sites, *Aedes vigilax* (Skuse), a known major vector of both RR and BF viruses, were scarce, comprising only 1.4% of the total collection (Hearnden and Kay 1995).

Based on 25 light trap collections during February and May 1991 at the Town Common and Residential, the total catch averaged 247 per night, with *Ae. vigilax* comprising 84.6%, *Cx. annulirostris* comprising 11.6%, and *An. amictus* and *Ae. normanensis* comprising a further 0.3%.

Alphavirus detection: The numbers of mosquitoes, pools, and isolates sorted by year and locality are given in Table 2. From the Ross River reservoir, 10 RR, one BF, and one Sindbis virus isolate were recovered from 39,403 mosquitoes (a further 5,316 in mixed locality pools or from minor sites were uninfected). Ross River virus was detected at all 4 lakeside localities and at Big Bay and Ross River in both 1991 and 1992. From 6,778 mosquitoes from the Town Common and Residential, one Sindbis virus isolate was detected.

Ross River virus was detected in 5 pools of *Ae. normanensis,* 4 of *An. amictus* (one of these was mixed with *An. annulipes*), and one of *Cx. annulirostris* (Table 3). Barmah Forest virus was isolated from *An. amictus* at Toonpan and 2 pools of *Cx. annulirostris* yielded Sindbis virus. All infected mosquitoes were recovered from collections during February or March of the wet seasons of 1990–91 (4 isolates from 7,150 mosquitoes) and 1991–92 (9 isolates from 16,327 mosquitoes). None was recovered from the wet season of 1992–93 (6,961 mosquitoes processed). Mosquito infection rates calculated using the formula of Chiang and Reeves (1962) indicated higher RR virus infection rates in *Ae. normanensis* during the wet season of 1991–92 (4.4–4.6 per 1,000) than 1990–91 (0.2–1.1 per 1,000). During the 1991–92 wet season, RR virus infection rates in *An. amictus* and *Cx. annulirostris* were 0.6–0.8 and 1.5 per 1,000, respectively.

Table 1. Extended.

1992			1993		
March	May–Oct.	Dec.	Feb.	April	Total
11 (2)	4 (3)		8 (1)		115 (5)
		5 (2)	28 (3)	2 (2)	73 (10)
3 (2)					21 (6)
					1 (1)
					10 (1)
6 (1)		9 (3)	123 (4)		223 (13)
1,156 (14)	24 (5)	1,065 (12)	1,896 (20)	3 (2)	9,700 (115)
			1 (1)	2 (1)	33 (7)
237 (4)	19 (11)	23 (4)	73 (3)		6,304 (96)
			6 (2)		165 (6)
5,440 (67)	3,512 (59)	495 (6)	374 (8)	398 (9)	14,721 (234)
2,597 (36)	1,272 (42)	70 (6)	361 (7)	1,094 (16)	8,733 (187)
	6 (3)		1 (1)		16 (9)
					604 (9)
1 (1)	185 (19)			5 (3)	602 (52)
				2 (1)	42 (7)
1,682 (18)	339 (20)	245 (4)	404 (5)	209 (6)	8,665 (132)
8 (3)	4 (1)	2 (1)		5 (1)	638 (24)
36 (3)	2 (1)		5 (2)	2 (1)	86 (12)
					9 (3)
					2 (1)
	1 (1)				1 (1)
	3 (1)		1 (1)		5 (3)
10 (2)	99 (4)	10 (1)		34 (3)	728 (38)
11,187 (153)	5,470 (169)	1,924 (39)	3,281 (58)	1,756 (45)	51,497 (991)

The 10 RR virus isolates detected by cell culture amplification and EIA were confirmed by RT-PCR. No other isolates were detected by RT-PCR.

Assessment of relative hazard: On the basis of 1991–93 data, the relative scores indicated that the hazard of infection (Table 4) was lowest at Big Bay (2), Antill Creek (2.8), and Ross River (7.5), and highest at Toonpan (14.6). No medically important alphaviruses were detected from the Town Common and Residential collections during February and May 1991.

DISCUSSION

The major objective of our project was to define whether there was a hazard associated with opening up parts of the reservoir for recreational use and, if so, to develop a practical firm of risk assessment achievable on minimal notice. The

Table 2. Mosquitoes collected by locality[1] and year with virus isolates in parentheses.

	Reservoir				
Year	Big Bay	Ross River	Antill Creek	Toonpan	Townsville
1990	57	205	69	400	
1991	3,208 (1)	4,970 (2)	2,307	736	6,778 (1)
1992	5,719 (1)	2,753 (3)	5,293 (1)	8,912 (4)	
1993	1,688	171	513	2,402	
Total	10,672 (2)	8,099 (5)	8,182 (1)	12,450 (4)	6,778 (1)
No. pools	225	177	172	204	83

[1] Does not include 5,316 mosquitoes collected from minor sites or in mixed pools.

Table 3. Alphaviruses isolated from mosquitoes collected from the Ross River reservoir and Townsville area, 1990–93.

Date[1] and code number	Virus	Collection site	Pool size and species	Mosquito infection rate/1,000
0291/45	Ross River	Ross River	100 *Ae. normanensis*	0.2
/83	Ross River	Big Bay	84 *Ae. normanensis*	1.1
0392/6	Ross River	Ross River	100 *Ae. normanensis*	4.4
/10	Ross River	Ross River	118 *Ae. normanensis*	4.4
/43	Ross River	Toonpan	119 *Ae. normanensis*	4.6
/47	Barmah Forest	Toonpan	100 *An. amictus*	0.3
/26	Ross River	Antill Creek	100 *An. amictus*	0.8
/44	Ross River	Toonpan	100 *An. amictus*	0.6
/49	Ross River	Toonpan	100 *An. amictus*	0.6
/53	Ross River	Ross River	117 *An. amictus*, *An. annulipes*	3.8
/59	Ross River	Big Bay	100 *Cx. annulirostris*	1.5
0291/40	Sindbis	Ross River	100 *Cx. annulirostris*	1.5
/76	Sindbis	Townsville	99 *Cx. annulirostris*	9.0

[1] 0291 refers to February 1991, etc.

problem of avian schistosome infection causing swimmer's itch has been addressed elsewhere (Hurley et al. 1994, 1995). This present paper specifically addresses alphavirus infections, which, from previous antibody studies, were shown to be common in the area (Barker-Hudson et al. 1993). This earlier work did not include consideration of another alphavirus, BF virus, which was first recognized as causing similar symptoms (i.e., rash, arthralgia, arthritis, and fever) in 1986 (Mackenzie et al. 1994). Although Sindbis virus infection is characterized by fever with vesicular rash elsewhere in the world, only subclinical infections have occurred in Australia (Russell 1995). Thus, this virus was not included in the final comparison.

Although it is recognized that both RR and BF viruses are catholic in their association with species of mosquitoes, reservoir-breeding *Cx. annulirostris* and *Ae. normanensis* are recognized as major vectors and there also have been isolates of both viruses from *An. amictus* and *An. annulipes* (Russell 1995). *Aedes vigilax,* whose breeding is mainly associated with the inundation of intertidal salt marsh, is also recognized as a major vector. However, this species constitutes a non-dam-associated hazard, as is evidenced by an epidemic polyarthritis incidence (RR virus is the etiological agent) of 350–425 cases/100,000 population (Communicable Diseases Intelligence 1994). Thus, our choice of key mosquito species was not solely based on abundance and known breeding in the trap localities and on RR and BF viruses detected from the adult mosquitoes collected from 1990 to 1993, but also on other factors known to influence vector potential, for example, host-feeding patterns including on humans, vector compe-

Table 4. Definition of relative hazard at different localities at the Ross River reservoir, based on mosquito species composition, abundance, and mosquito infection rates (MIR).

	Locality				
Criterion	Big Bay	Ross River	Antill Creek	Toonpan	Townsville
Mosquitoes					
% vectors[1]	47.8 (1)	72.0 (1.5)	82.4 (1.72)	88.0 (1.84)	96.5 (2.0)
Mean catch/night	72.4 (1)	72.6 (1)	116.5 (1.61)	191 (2.64)	247 (3.42)
Virus					
MIR/1,000	0.2 (2)	0.5 (5)	0.1 (1)	0.3 (3)	0 (1)
Relative hazard	2	7.5	2.8	14.6	6.8

[1] Based on percent of total catch of *Ae. normanensis, An. amictus,* and *Cx. annulirostris* for reservoir sites. For Townsville, *Ae. vigilax* is included. Numbers in parentheses indicate a comparative scale in relation to the lowest score for each parameter.

tence, and dispersal. Analysis of population age structure was not considered to be a viable option because of its labor intensiveness and because the 2 major recognized vectors, *Cx. annulirostris* and *Ae. vigilax,* were thought to be panmictic throughout the entire study area and certainly within the 4 reservoir localities. Furthermore, RR virus has also been shown to be transmitted vertically (Russell 1995). Because there was uncertainty about the mosquito source of the Ross River isolate 0392/53, *An. annulipes* was not included in assessment of hazard. Conversely, because 4 other isolates were derived from *An. amictus,* it was included with the recognized vectors *Cx. annulirostris, Ae. normanensis,* and *Ae. vigilax.*

We believe that the EIA system employed for detection of alphavirus antigens in mosquitoes is a rapid, sensitive, and practical means of surveillance. On the basis of detection of RR virus from 60 pools, this assay proved as sensitive as the RT-PCR. These findings essentially support similar comparisons between the EIA and traditional cell culture methods using both field- and laboratory-infected mosquito pools (Oliveira et al. 1995).

Hearnden and Kay (1995) have shown that the period of maximum mosquito abundance at the dam corresponds with the onset of the wet season, normally in January and extending into the post-wet season month of May. Populations were generally depressed during the cooler months of June to August and from September, nonaedine species began to increase. *Aedes normanensis,* which was particularly abundant at Toonpan, was more ephemeral than the other major species and usually occurred, sometimes in plague proportions, following wet-season rainfall. Thus, both our data on mosquito abundance and on alphavirus activity would indicate that the hazard is greater during this period.

In terms of localities within the Ross River reservoir that offer acceptable low infection risk for recreational development, Big Bay was ranked the safest in terms of a lower mosquito abundance as well as lower prevalence of known vectors and infection rates. Numbers of *Cx. annulirostris* were similar at the 4 localities within the reservoir (Hearnden and Kay 1995) but *Ae. normanensis* and *An. amictus* were fewer at Big Bay because major breeding of these species probably occurs at the opposite end of the reservoir at Toonpan and Antill Creek. However, more precise definition of breeding sites is required. At Big Bay, the high prevalence of *An. annulipes* was associated with extensive breeding in nearby floating *Hydrilla verticillata* beds (Hearnden and Kay, unpublished data).

Although our assessment indicates that Big Bay is a prime site for recreational development, the limitations of such an approach are also recognized. Why, for example, were alphavirus infection rates greatest in the failed wet season of 1991–92 compared to the other 2 years? This is not simply related to numbers of mosquitoes processed. In failed wet seasons, the reservoir does not fill to capacity and as a consequence, leaves broad expanses of suitable breeding habitat for *Ae. normanensis* and *An. amictus* in the Toonpan and Antill Creek areas. In good wet seasons, these areas would be quickly inundated. Thus, the hazard of alphavirus infection at different points of the Ross River reservoir could vary according to seasonal conditions.

Our evaluation of alphavirus activity at 2 localities around urban Townsville was limited to collections during the wet and post-wet season of 1990–91 and was based on only 6,778 mosquitoes. In view of an accepted poor notification rate and subclinical to clinical expression of 80:1 for epidemic polyarthritis (Kay and Aaskov 1988) as well as our data, we would suggest that an excursion to the Ross River reservoir does not present any greater hazard of infection than that which occurs in day-to-day living. This is especially true as most of the planned recreational activity will be diurnal, when these mosquito species are inactive. However, because the stage 2A lake is still undergoing ecological change, periodic surveillance is required.

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