

# NEW RECORDS OF ARBOVIRUSES ISOLATED FROM MOSQUITOES IN THE NORTHERN TERRITORY, 1982-1992

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## INTRODUCTION

The isolation of arboviruses from mosquitoes in the NT began in April 1974 with the collection of mosquitoes by NT Health to determine the vectors of Murray Valley encephalitis virus following an Australia wide encephalitis epidemic. This limited collection yielded Sindbis virus and the then newly recognised Leanyer virus (Doherty et al. 1977).

Between October 1974 and May 1976, a study to determine the vector(s) of bovine ephemeral fever virus was the first longitudinal study of arboviruses and vectors in the NT (Standfast et al. 1984). Ten arboviruses were isolated from 5 species of mosquitoes from 57,596 mosquitoes. However, the isolations were all made from a single locality near a sentinel herd of cattle pastured at Beatrice Hill, 60km east of Darwin.

Approximately 23,000 mosquitoes collected by NT Health between 1979 and 1981 from localities including Darwin, Jabiru, Nhulunbuy and Katherine were sent to the Victorian Institute of Animal Science and to the University of Western Australia for virus isolation but no viruses were isolated. Between 1979 and 1981, blood samples requested for virus isolation by NT Health from sentinel cattle between Darwin and Alice Springs was sent to Queensland Health for tests for haemagglutination inhibition (HI) antibody to flaviviruses. The results indicated widespread flavivirus activity (Whelan and Gard 1981). However, inability of serologic tests to define precisely the infecting agent prompted a decision to collect mosquitoes in the NT and to isolate viruses from them.

A comprehensive collection of mosquitoes for arbovirus isolation was begun in 1982, with identifications carried out in the newly established A.L. Rose Arbovirus Laboratory under a collaborative program between NT Health and the Department of Primary Industry. Selection of collection sites was based on a human health perspective and were primarily in and near major population centres, but also included locations considered to be ecologically suitable for arbovirus transmission. All isolates were retained for later identification with the view that some might comprise new arboviruses with human and animal health implications.

Identifications of alphaviruses and flaviviruses to 1992 have been reported (Whelan and Weir 1993). A total of 342,737 mosquitoes collected yielded 117 isolates of 5 different alphaviruses and flaviviruses from a total of 6 species of mosquitoes collected from Darwin to Ti Tree, 1400 km south of Darwin. However, most of the other 231 viruses remained unidentified. This paper identifies these latter viruses.

## MATERIALS AND METHODS

### Mosquito Collection and Virus Isolation

Methods were as described previously by Whelan and Weir (1993). Mosquitoes were trapped using EVS CO<sub>2</sub> baited light traps and transferred to the laboratory for speciation. Mosquitoes were sorted into pools of not more than 50 individuals of a single species and stored in liquid nitrogen prior to transfer for virus isolation.

Virus isolation during 1982-1983 used 3 cell lines, BHK-21, HmLu-1 and Vero. C6/36 were introduced as a first passage prior to inoculation to mammalian cells in 1984, and the Vero cell line was replaced with the CV-1 cell line. After 1986, Vero cells were removed from the isolation system completely as described (Whelan and Weir 1993).

## VIRUS IDENTIFICATION

### Serotyping

Virus isolates from 1982-1992 were initially screened using the plaque reduction neutralization test (PRNT) against antibodies produced to members of the bovine ephemeral fever (BEF), bluetongue (BLU), epizootic haemorrhagic disease (EHD), Palyam (PAL), Simbu (SIM), Group A (alphaviruses) and Group B (flaviviruses) serogroups. Isolates not neutralized were then screened against a panel of antibodies (Table 1) using an indirect immunofluorescence antibody test (IFA) (Zeller et al. 1989) and serogrouping enzyme-linked immunosorbent assay (SG-

ELISA) (Blacksell et al. 1995). IFA was read using a scale of 0 (negative) to 4+ (maximum intensity). SG-ELISA optical densities were measured and a difference of >0.2 between control and test wells was considered positive. Isolates not reacting by IFA were grouped according to location, date, source species, time to produce cytopathic effect (CPE) and cell lines producing CPE. Hyperimmune mouse antibody was prepared (Brandt et al. 1967) to represent members of these groups for further IFA and SG-ELISA testing.

Isolates placed in recognised serogroups were typed by a micro-neutralisation test (Corner et al. 1995) using specific hyperimmune mouse serum prepared to prototype isolates.

**Table 1.** Antisera used for indirect immunofluorescent antibody (IFA) grouping identification tests of NT isolates

**Grouping fluids:** Groups A, C, bluetongue, Bunyamwera, California, Capim, Guama, Kemerovo, Patois, Phlebotomus fever, Simbu, Tacaribe, vesicular stomatitis. Polyvalent fluids: 1 (Bahig, Berg el Arab, EgAn 1398-61, Matruh, Matari Tete), 2 (Alajuela, Belem, Gamboa, Jurona, Minatitlan), 3 (Bakau, Ketapang, Koongol, Mapputta, Maprik, Trubanam Wongol), 4 (Grand Arbaud, Nyamanini, Thogoto, Uukuniemi), 5 (Hughes, Lone Star, Matucare, Sawgrass, Soldado), 6 (Chaco, Marco, Pacui, Timbo), 7 (Flanders, Hart Park, Kern Canyon, Klamath, Mount Elgon bat), 8 (bluetongue Changuinola, Colorado tick fever, epizootic haemorrhagic disease, IbAr22619, Irituia), 9 (Aruac, Navarro, Patois, Trinité), 10 (Dera Ghazi Khan, Dhori, Upolu, Wanowrie), 11 (Anopheles A, Lukuni, Tacaiuma, CoAr 1071, CoAr 36), 12 (Anopheles B, Boraceia, M'Poko, Turlock, Umbre), 13 (Bobia, Olifantsvlei, Okola, Tataguine, Witwatersrand), 14 (Bwamba, Eretmapodites 147, Kamese, Mossuril, Nyando, Pongola), 15 (Bhanja, Congo, Dugbe, Ganjam, Hazara), 16 (Acado, Corriparta, d'Aguilar, Eubenangee, Kasba, Palyam, Pata, Vellore), 17 (Bandia, Johnston Atoll, Kaisodi, Lanj Quaranfil, Qalyub, Silverwater), 18 (rabies, herpes 1, lymphocytic choriomeningitis, Newcastle disease, vaccinia).

**Arenaviridae:** (ungrouped) Johnston Atoll.

**Reoviridae:** *Orbivirus* (Corriparta) Corriparta, (Epizootic haemorrhagic disease) EHD (New Jersey), (Eubenangee) Eubenangee, (Kemerovo) Nugget, (Palyam) D'Aguilar, Bunyip Creek, CSIRO Village, DPP 66, Marrakai, (Wall Wallal, (Warrego) Warrego, (Wongorr) Wongorr, (ungrouped) Japanaut, Mitchell River, Lake Clarendon.

**Rhabdoviridae:** *Lyssavirus* (Bovine ephemeral fever) Adelaide River, Berrimah, bovine ephemeral fever, Kimberley Malakal, (Rabies) Charleville, (Tibrogargan) Ngaingan, Tibrogargan, (ungrouped) Almpiwar, Joinjakaka, Koolpinyah, Kununurra, Parry Creek.

**Bunyaviridae:** *Bunyavirus* (Koongol) Koongol, Wongol, (Simbu) Aino, Douglas, Peaton, Shuni, Tinaroo, *Nairovirus* (Sakhalin) Taggart, *Phlebovirus* (Uukuniemi) Precarious Point, (Mapputta) Gan Gan, Mapputta, Maprik, Trubanam, (ungrouped) Belmont, Kowanyama, Upolu.

**Flaviviridae:** *Flavivirus* (B) Alfuy, Edge Hill, Gadgets Gully, Kokobera, Kunjin, Murray Valley encephalitis, Saumarez Reef, Sepik, Stratford.

**Togaviridae:** Alphavirus (A) Barmah Forest, Bebaru, Getah, Ross River, Semliki Forest, Sindbis, Whataroa.

## Electron microscopy

Representative unidentified viruses were inoculated onto confluent cells in a 25 cm<sup>2</sup> tissue culture flask and sterile 200 mesh gold, Parlodion/carbon coated grids seeded with 1 x 10<sup>5</sup> Vero and BHK-21 cells respectively. Grids were removed each 24 h after inoculation and examined by electron microscopy as described by Hyatt et al. (1987). When CPE reached 50%, cells from a 25 cm<sup>2</sup> flask were pelleted for 10 min at 2000rpm, fixed in 2.5% glutaraldehyde, post fixed in 1% osmium tetroxide, dehydrated through graded ethanol and embedded in Spurr's resin in preparation for staining of thin sections. Specimens were examined using a Hitachi H600 scanning transmission electron microscope and a JEOL JEM 1200 transmission electron microscope.

## RESULTS

A total of 342,737 mosquitoes were tested for virus between 1982 and 1992. Viruses are listed in Tables 2, 3 by month of collection, location and year for 1982-1992. Eleven mosquito species collected from 13 localities yielded a total of 175 orbiviruses (Table 5), 14 members of the family Bunyaviridae, 1 member of the family Rhabdoviridae (Table 6), 7 bunyavirus-like, 24 orbivirus-like, 1 rhabdovirus-like and 9 unidentified viruses (Table 7).

**Family Reoviridae, genus Orbivirus**

**Wongorr (WGR) serogroup:** 129 Wongorr serogroup members [Wongorr (WGR), Paroo River (PR), Picola (PIA)] were isolated from 9 mosquito spp. (Table 5) collected at Darwin (WGR 20, PIA 6, PR 11, WGR Group 14), Darwin Rural (PR 1, WGR Group 2), Katherine (WGR 23, PIA 15, PR 13, WGR Group 11), Larrimah (PR 1), Mataranka (WGR 1, PR 2, WGR Group 1), Kakadu (WGR 1) and numerous localities along the Arnhem Highway (WGR 1, PIA 1, WGR Group 4) (Table 3).

**Wallal (WAL) serogroup:** 9 isolates were obtained from *Cx. annulirostris* (1) and *Ae. normanensis* (8) mosquitoes collected at Jabiru(1), Larrimah (2), Mataranka (4) and Katherine (2). One of the 2 isolates from Larrimah was identified as Mudjinbarry (MUD) virus, while single isolates from Larrimah and Mataranka were only identified to serogroup. The remaining 6 isolates were Wallal serotype (Tables 3,5).

**Warrego (WAR) serogroup:** 10 isolates from *Cx. annulirostris* (6), *Ae. normanensis* (3) and *An. annulipes* (1) were collected at Jabiru (1), Larrimah (1), Mataranka / Larrimah (1), Katherine (6) and the Darwin rural area (1). No attempt was made to type this serogroup (Tables 3,5).

**Eubenangee (EUB) serogroup:** EUB virus was isolated on 4 occasions from 3 mosquito species, *Ae. lineatopennis* (1), *Ae. vigilax* (1) and *Cx. annulirostris* (2) collected from Darwin (3) and Katherine (1) (Tables 3,5).

**Corriparta (COR) serogroup:** Corriparta virus was isolated on 18 occasions from *Ae. vigilax* (1), *Cx. annulirostris* (13), *Ae. normanensis* (2), *An. novaguinensis* (1) and *An. amictus* (1) collected from Darwin (6), Mataranka (3), Palumpa (7) and the Darwin rural area (2) (Tables 3,5).

**Bluetongue (BLU) serogroup:** 4 isolates of bluetongue serotype 16 were isolated from 2 species at 2 locations. These isolates were from *Ae. vigilax* (1) and *Cx. annulirostris* (3) collected at Darwin and the Darwin rural area.

**Palyam (PAL) serogroup:** CSIRO Village virus was isolated once *Cx. annulirostris* collected at Darwin (Tables 3,5).

**Orbivirus-like viruses:** 24 isolates were divided into 2 serologically distinct groups by IFA. The first group contained 22 members, 20 of which were isolated from *Cx. annulirostris* collected at Darwin, Katherine, Larrimah and localities along the Arnhem Highway (DPP-612, -632, -643, -1009, -1164, -1170, -1447, -1660, -1671, -1680, -1684, -1687, -1688, -1690, -1700, -1705, -1709, -1719, and -1724). The remaining 2 were isolated from *Ae. normanensis* from Katherine and Mataranka (DPP-628, -611). The second group contained 2 viruses (DPP-650, -653) which were isolated from *Ae. normanensis* collected at Mataranka in 1984 (Tables 3,4,5). Electron microscopy (EM) of representative group members has shown icosahedral naked spherical particles of 60-75nm diam, with electron dense centres and an fibrillar outer coat. Cellular ultrastructural changes show inclusion bodies, virus tubules and virus budding through the plasma membrane. The size and shape of the virus and ultrastructural changes produced by the virus are indicative of an orbivirus infection (Hyatt et al.1987).

**Family Bunyaviridae**

**Mapputta serogroup:** 3 Mapputta (MAP) serogroup isolates were obtained from 5 species of mosquito collected at Darwin and Katherine: Trubanaman (TRU) virus on 4 occasions from *An. meraukensis* and once from *An. annulipes*; Mapputta virus on 1 occasion from *An. farauti* collected at Darwin; Gan Gan (GG) virus on one occasion from *Ae. normanensis* at Katherine; and 1 Mapputta serogroup virus (not further typed) from *Cx. annulirostris* at Katherine (Tables 3,6).

**Leanyer virus:** Preliminary testing failed to place this isolate serologically. Electron microscopic examination of this isolate from *An. meraukensis* at Darwin, 1983 showed 100-120nm spherical enveloped particles within and budding from Golgi bodies. Examination by the Grid Cell Culture Technique (GCCT) showed 120nm virions budding through the plasma membrane and 100-120nm pleomorphic virus attached to the grid substrate. Subsequent neutralisation tests showed this isolate to be Leanyer virus.

Other morphologically similar isolates were tested and found to be Kowanyama (KOW) virus on 4 occasions from *An. meraukensis* collected at Darwin, and Yacaaba virus from a pool of *Ae. normanensis* collected at Mataranka.

**Bunyavirus-like viruses:** 7 isolates were shown by IFA and neutralisation to be strains of 2 serologically distinct viruses. The first virus is represented by 5 isolates (DPP-212, -382, -396, -400, and -1182) isolated from *An. meraukensis*, *An. annulipes* and *An. amictus* collected at Darwin and Palumpa. The second virus is represented by 2 isolates (DPP-186

and, -646) isolated from *An. meraukensis* and *An. normanensis* collected at Darwin and Mataranka (Tables 3,7).

Thin section electron microscopy of each virus showed spherical, enveloped particles 90-100nm diam closely associated with Golgi bodies in the cell cytosol. Particles also were observed within cellular vesicles and in intracellular spaces. EM showed pleomorphic virions of 100nm diam, indicating a bunyavirus.

### Family Rhabdoviridae

Oakvale (OAK) virus was isolated once from a pool of *Ae. vigilax* collected at Darwin in January 1984 (Tables 2,3,4).

**Rhabdovirus-like viruses:** 1 rhabdovirus-like isolate was obtained from *Cx. annulirostris* collected at Darwin, February 1987 (Tables 2,3,7). Electron microscopy showed bullet shaped particles approximately 150 x 75nm with a distinct envelope and a helical core budding through the plasma membrane of the infected cells.

### Unidentified viruses

Nine virus isolates were not identified. They were isolated from *Cx. annulirostris* (5), *An. amictus* (1), *An. normanensis* (1), *Ae. vigilax* (1) and *An. hilli* (1) collected at Darwin, Katherine and the Darwin rural area (Tables 3, 7).

## DISCUSSION

Wongorr virus has previously been isolated from *Cx. annulirostris* and *Ae. lineatopennis* mosquitoes (Doherty et al. 1973,1979). Parkes (1995) has confirmed that Picola (PIA) and Paroo River (PR) viruses (Marshall et al. 1987) are orbiviruses most closely related to Wongorr virus (Zeller et al. 1989). Previously isolated from western NSW, PIA virus from *Ae. normanensis*, *An. farauti* and *An. meraukensis*, and PR virus from *Ae. lineatopennis* and *An. normanensis* collected in the NT significantly increases the known hosts and ranges of these 2 viruses. Wongorr virus (Doherty et al. 1973, Standfast et al. 1984, Liehne et al. 1981) had been isolated from *Ae. lineatopennis*, *C. annulirostris* and *Ae. normanensis*, respectively. The isolations of WGR virus from *Ae. notoscriptus*, *Ae. vigilax*, *An. amictus* and *An. annulipes* more than doubles the known host range for this virus. Thirty three WGR serogroup viruses that were not identified as either WGR, PIA or PR were isolated from *Ae. normanensis*, *An. meraukensis*, *Ae. reesi*, *C. annulirostris* and *Ae. vigilax*. Testing of these viruses using IFA produced a 2+, 3+ or 4+ result when reacted with WGR serogroup antibody. Wallal and Mudjinbarry (MUD) viruses have been isolated from *Culicoides* sp. and mosquito collected in the NT (Doherty et al. 1973, 1978 and Standfast et al. 1984). Isolation of WAL virus on 5 occasions from *Ae. normanensis* and once from *Cx. annulirostris* and MUD virus from *Ae. normanensis*, suggests that *Ae. normanensis* is a common mosquito host of these viruses. These isolates were from the more arid regions of the NT (Jabiru, Katherine, Larrimah and Mataranka). Two WAL serogroup viruses were not neutralized with antibody prepared to the WAL and MUD prototype strains and may be new viruses within the WAL serogroup.

Isolations of WAR group virus have been made from *Culicoides* (Doherty et al. 1973, Standfast et al. 1984) and *Cx. annulirostris* (Doherty et al. 1973). Between 1983 and 1992, 10 WAR serogroup members were isolated from mosquitoes belonging to 3 species (*Ae. normanensis*, *An. annulipes* and *Cx. annulirostris*). *Aedes normanensis* was the species from which most isolates were obtained. *Aedes normanensis* and *An. annulipes* are new host species for WAR serogroup viruses.

Standfast et al. (1984) isolated EUB serogroup members from *Cx. annulirostris* and *An. farauti*, and Doherty et al. (1968) isolated this virus from a mixed pool of mosquitoes. We obtained 4 EUB virus isolates, 2 from *Cx. annulirostris* and 1 each from *Ae. lineatopennis* and *Ae. vigilax*, the latter 2 being new host species.

*Culex annulirostris* is the species from which Corripata (COR) virus was isolated most frequently, on 13 occasions, during the period 1982-1988, in an area from Darwin, south to Mataranka and west to Peppimenarti. Corripata virus was also isolated from *Ae. normanensis* on 2 occasions and from *Ae. vigilax*, *An. amictus* and *An. novaguinensis* once each. Isolations from *Ae. normanensis*, *Ae. vigilax*, *An. amictus* and *An. novaguinensis* are new mosquito records for this virus serogroup.

Four bluetongue (BLU) type 16 isolates were made during the period 1990-1992 from *Cx. annulirostris* and *Ae. vigilax* collected in February-April. All isolates were from the Darwin or Darwin rural area and are of particular interest as no isolates of BLU were made from sentinel animals in 1990. These are the first BLU isolations from mosquitoes in Australia, although BLU viruses have been isolated from mosquitoes in Indonesia (Sendow et al. 1994 and Brown et al. 1992). These species of mosquitoes should be regarded as potential vectors of BLU virus and suggests that a vector competence study is warranted.

CSIRO Village virus was obtained from *Cx. annulirostris* mosquitoes collected at Darwin.

Eight Mapputta serogroup members were isolated: GG (1), MAP (1), TRU (5) and 1 isolate which could not be neutralized using homologous antibody prepared to these 3 serotypes. This unidentified virus is not an isolate of MRK virus, but possibly a newly recognised member of the MAP serogroup. TRU virus was isolated from *An. meraukensis*,

MAP virus isolated from *An. farauti* and the untyped MAP serogroup virus isolated from *Cx. annulirostris* mosquitoes. These are new records for these viruses in the NT. GG virus has been implicated with human disease in New South Wales but not in the NT.

Originally isolated from *An. amictus* and *An. annulipes* collected at Mitchell River (Kowanyama) in 1963 (Doherty et al. 1968), Kowanyama virus was isolated on 4 occasions from *An. meraukensis* collected at Darwin during 1982-1983. No other isolations of this virus have been made in the NT. Changes in the isolation system in 1984 may account for this occurrence. The mosquito species from which this virus was isolated is a new host record and the isolate from the NT extends the recognised geographical distribution of the virus.

Yacaaba virus was originally isolated from *Ae. vigilax* collected at Nelson Bay, New South Wales, 1970 (Gard et al. 1973). The first NT isolate of this virus was from *Ae. normanensis* collected at Mataranka in March 1984. This finding increases the recognised geographical distribution and host range of Yacaaba virus.

First isolated by Doherty in 1974 (Doherty et al. 1977) from *An. meraukensis* and subsequently from *Culicoides* sp. by Standfast from 1974-1976 (Standfast et al. 1984), Leanyer virus was isolated once from *An. meraukensis* collected at Darwin, 1983.

The identification of Oakvale virus from *Cx. annulirostris* mosquitoes in the NT increases the recognised geographic distribution and host range of this virus.

Orbivirus-like, bunyavirus-like and rhabdovirus-like viruses have been tested extensively against antibodies prepared to a comprehensive battery of viruses (Table 1). Further serological testing using antibodies to each of the known Australian viruses has not produced information useful for placing any of these in a family. These isolates are undergoing further characterization with the intent of providing such placement.

## Conclusion

A total of 190 viruses from the families Reoviridae, Bunyaviridae and Rhabdoviridae as well as 41 unidentified viruses were recovered from 342,737 mosquitoes collected between 1982-1992. High isolation rates of viruses from 12 species of mosquitoes collected in most population centres of the NT indicate a high level of arbovirus activity. The number of possible vectors, some previously unrecorded, and the number of new strains of serologically unique viruses supports this observation. This study being the second longitudinal study of arboviruses in the NT, affirms the work of Standfast et al. (1984), increases the known range of several arboviruses, and demonstrates that there are a considerable number of mosquito species that carry viruses. These arboviruses may have the potential to cause disease in humans and in livestock.

Disease of unknown origin, including those with possible viral aetiologies, is a growing concern in the medical community. Reagents prepared from the 41 unidentified isolates may be useful in determining the aetiology of certain of these undiagnosed illnesses. Disease of livestock in the NT, with stock being mustered once or twice per year, may be underestimated. Sick animals may not be observed at all and then not unless clinical signs are obvious or the numbers affected are high. Consideration should be given to mosquitoes as potential vectors of bluetongue virus. This research is the result of a comprehensive surveillance system which we suggest should be maintained to monitor not only alphavirus and flavivirus illnesses of humans but also to define the arboviral aetiology of other clinical illnesses of humans, livestock, and wild animals.

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**Table 2.** Total viruses (number of isolates) from mosquitoes collected in the NT 1982-92, by month.

Month:	January	February	March	April	May	June	December
	WGR (2)	WGR (2)	WGR (19)	WGR (4)	PR (2)	WGR (1)	WGR GP (2)
	PIA (2)	PIA (12)	PIA (5)	PIA (3)	WGR GP (1)	WAL (1)	
	PR (4)	PR (15)	PR (6)	WGR GP (2)	KOW (2)	WAR (1)	
	WGR GP (1)	WGR GP (17)	WGR GP (10)	KOW (2)	LEAV (1)		
	PAL (1)	EUB (1)	EUB (1)	EUB (1)	EUB (1)		
	COR (1)	COR (2)	COR (3)	COR (12)	TRU (1)		
	WAL (1)	WAR (7)	TRU (3)	TRU (1)	BUNYA (1)		
	OAK (1)	WAL (4)	MAP (1)	GAN (1)	ORBI (1)		
	ORBI (1)	WAL GP (1)	WAR (2)	YAC (1)	UNIDENT(2)		
	BLU 16 (1)	WAR GP (1)	MUD (1)	WAL GP (1)			
		BLU 16 (1)	BLU 16 (1)	BLU 16 (1)			
		ORBI (16)	BUNYA (4)	BUNYA (2)			
		RHABDO (1)	ORBI (4)	ORBI (2)			
		UNIDENT (1)	UNIDENT (2)	UNIDENT (1)			
<b>Mosquitoes collected</b>	30,881	69,506	69,123	52,863	60,124	26,859	5,734

**Table 3.** Total viruses (number of isolates) from mosquitoes collected in the NT 1982-92, by town.

Location:	Darwin	Dwn Rural	Kakadu	Katherine	Larrimah	Mataranka	Arnhem Hwy	Nhulunbuy	Palumpa
	WGR (20)	WGR GP (2)	WGR (1)	WGR (23)	PR (1)	WGR (1)	WGR (1)	WGR (1)	COR (7)
	PIA (6)	COR (2)		PAI (15)	WAL (1)	PR (2)	PAI (1)	WGR GP (1)	BUNYA (1)
	PR (12)	WAR (1)		PR (12)	WAL GP (1)	WGR GP (1)	WGR GP (4)	UNIDENT (1)	
	WGR GP (14)	UNIDENT (1)		WGR GP (11)	WAR (2)	COR (3)			
	EUB (3)	BLU (16)		WAL (1)		MUD (1)			
	COR (6)			WAL GP (1)		WAL (3)			
	PAL (1)			WAR (6)		YAC (1)			
	KOW (4)			GAN (1)		BUNYA (1)			
	LEAV (1)			MAP GP (1)		ORBI (4)			
	OAK (1)			EUB (1)					
	TRU (5)			ORBI (17)					
	MAP (1)			UNIDENT (5)					
	BLU 16 (3)								
	BUNYA (5)								
	ORBI (3)								
	RHABDO (1)								
	UNIDENT (2)								
<b>Mosquitoes collected</b>	194,623	18,761	38,490	31,788	2,992	34,334	6,323	6,403	4,983

**Table 4.** Total viruses (number of isolates) from mosquitoes collected in the NT 1982-1992, by year.

Year:	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992
	WGR(1)	WGR(1)	WGR(11)	PR(1)	WGR(6)	WGR(1)	WGR(3)	WGR(17)	WAR(1)	WGR(2)	WGR(6)
	PIA(3)	PR(1)	PIA(1)	WGR GP(2)	PR(3)	PR(3)	PIA(1)	PIA(16)	UNDENT(1)	PIA(1)	PR(1)
	PR(2)	WAL(1)	PR(5)	WAL(2)	WGR GP(2)	WGR GP(1)	PR(1)	PR(9)	BLU 16(1)	PR(1)	WGR GP(4)
	WGR GP(1)	WAR(1)	WGR GP(3)	WAL GP(1)	COR(1)	WAL(2)	WGR GP(2)	WGRGP(17)		WGRGP(1)	WAR(1)
	KOW(1)	COR(1)	WAL(1)	COR(2)	ORBI(1)	COR(9)	COR(2)	WAR(4)		BLU 16(1)	EUB(1)
	MAP(1)	EUB(3)	MUD(1)	PAL(1)		BUNYA(1)	ORBI(1)	MAP GP(1)			UNIDENT(3)
	TRU(3)	KOW(3)	WAR(3)			ORBI(2)		ORBI(13)			BLU 16(1)
	BUNYA(2)	LEAV(1)	COR(3)			RHABDO(1)		UNIDENT(2)			
		TRU(2)	YAC(1)								
		GAN(1)	OAK(1)								
		BUNYA(3)	BUNYA(1)								
		UNIDENT(1)	ORBI(7)								
			UNIDENT(2)								
<b>Mosquitoes collected</b>	76,650	49,642	37,458	13,155	22,651	30,883	24,968	25,746	9,706	14,160	37,718



Table 5. Mosquito species from which orbiviruses were isolated.

Mosquito species:	Wongorr serogroup				Wallal serogroup								TOTAL
	WGR	PIA	PR	Gp.	WAL	MUD	Gp.	WAR	EUB	COR	BLU	PAL	
<i>Ae. lineatopennis</i>	-	-	1 <sup>ab</sup>	-	-	-	-	-	1 <sup>a</sup>	-	-	-	2
<i>Ae. normanensis</i>	2	2 <sup>ab</sup>	4 <sup>ab</sup>	2 <sup>ac</sup>	5 <sup>a</sup>	1	2 <sup>ac</sup>	3 <sup>a</sup>	-	2 <sup>a</sup>	-	-	23
<i>Ae. notoscriptus</i>	-	-	-	1 <sup>ac</sup>	-	-	-	-	-	-	-	-	1
<i>Ae. reesi</i>	-	-	-	1 <sup>ac</sup>	-	-	-	-	-	-	-	-	1
<i>Ae. vigilax</i>	4 <sup>a</sup>	1	-	2 <sup>ac</sup>	-	-	-	-	1 <sup>a</sup>	1 <sup>a</sup>	1 <sup>ac</sup>	-	10
<i>An. farauti</i>	-	1 <sup>ab</sup>	-	-	-	-	-	-	-	-	-	-	1
<i>An. amictus</i>	-	-	-	-	-	-	-	-	-	1 <sup>a</sup>	-	-	1
<i>An. annulipes</i>	1 <sup>a</sup>	-	-	-	-	-	-	1 <sup>a</sup>	-	-	-	-	2
<i>An. meraukensis</i>	-	2 <sup>ab</sup>	-	1 <sup>ac</sup>	-	-	-	-	-	-	-	-	3
<i>An. novaguinensis</i>	-	-	-	-	-	-	-	-	-	1 <sup>a</sup>	-	-	1
<i>Cx. annulirostris</i>	40	16 <sup>b</sup>	22 <sup>b</sup>	26 <sup>ac</sup>	1 <sup>a</sup>	-	-	6	2	13	3 <sup>ac</sup>	1	130
<b>Total</b>	<b>47</b>	<b>22</b>	<b>27</b>	<b>33</b>	<b>6</b>	<b>1</b>	<b>2</b>	<b>10</b>	<b>4</b>	<b>18</b>	<b>4</b>	<b>1</b>	<b>175</b>

<sup>a</sup>New host species for this virus<sup>b</sup>New record for the Northern Territory<sup>c</sup>New record for Australia

Table 6. Mosquito species from which members of the Bunyaviridae and Rhabdoviridae were isolated.

Mapputta serogroup viruses									
Mosquito species:	TRU	MAP	GG	Gp.	LEAV	YAC	KOW	OAK	TOTAL
<i>Ae. normanensis</i>	-	-	1 <sup>ab</sup>	-	-	1 <sup>ab</sup>	-	-	2
<i>Ae. vigilax</i>	-	-	-	-	-	-	-	1 <sup>ab</sup>	1
<i>An. farauti</i>	-	1 <sup>a</sup>	-	-	-	-	-	-	1
<i>An. annulipes</i>	1	-	-	-	-	-	-	-	1
<i>An. meraukensis</i>	4 <sup>a</sup>	-	-	-	1	-	4 <sup>ab</sup>	-	9
<i>Cx. annulirostris</i>	-	-	-	1 <sup>ac</sup>	-	-	-	-	1
Total	5	1	1	1	1	1	4	1	15

Table 7. Mosquito species from which unidentified viruses were isolated.

Mosquito species:	Bunyavirus-like	Orbivirus-like	Rhabdovirus-like	Unidentified	TOTAL
<i>Ae. normanensis</i>	1 <sup>ac</sup>	4 <sup>ac</sup>	-	1 <sup>ac</sup>	6
<i>Ae. vigilax</i>	-	-	-	1 <sup>ac</sup>	1
<i>An. amictus</i>	1 <sup>ac</sup>	-	-	1 <sup>ac</sup>	2
<i>An. annulipes</i>	1 <sup>ac</sup>	-	-	-	1
<i>An. meraukensis</i>	4 <sup>ac</sup>	-	-	-	4
<i>Cx. annulirostris</i>	-	20 <sup>ac</sup>	1 <sup>ac</sup>	5 <sup>ac</sup>	26
<i>An. hilli</i>	-	-	-	1	1
Total	7	24	1	9	41

<sup>a</sup>New host species for this virus

<sup>b</sup>New record for the Northern Territory

<sup>c</sup>New record for Australia

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