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Mudjinbarry Virus, an Orbivirus Related to Wallal Virus Isolated from Midges from the Northern Territory of Australia

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Abstract

A virus strain (NT14952) isolated from *Culicoides marksi* collected in the Alligator Rivers area of the Northern Territory of Australia in September 1971 was shown to be an orbivirus antigenically related to but distinct from Wallal virus. The name 'Mudjinbarry virus' is suggested for it.

Introduction

Seven viruses have been isolated from *Culicoides* midges in Australia (Doherty 1972), four of them identified by electron microscopy and physicochemical characteristics as orbiviruses (Schnagl and Holmes 1971; Gorman and Lecatsas 1972; Lecatsas and Gorman 1972). One of the orbiviruses, Wallal virus, was isolated from *Culicoides dycei* and *Culicoides marksi* collected at Charleville, Qld, in 1970 (Doherty *et al.* 1973). No antigenic relationship has been detected between it and any other virus (Berge 1975). The present paper describes the isolation of a related but distinct virus from midges collected in the Northern Territory during the reconnaissance phase of a study of insects attacking livestock in the 'top end' of the Northern Territory, where large numbers of feral buffalo (*Bubalus bubalis*) are found.

Materials and Methods

Mosquitoes and midges were collected by light trap, truck trap or on bait cattle or buffalo at Mudginberri Station, Mudjinbarry, in the Alligator Rivers region of the Northern Territory (latitude 12°36′S., longitude 132°52′E.) by A.L.D. and H.A.S. in March–April and September 1971. Techniques of collection, handling, transport and storage have been described (Dyce *et al.* 1972). Some bait animals were tranquillized by techniques described by Keep (1973).

Arthropods processed for virus isolation were suspended in 0·75% bovine serum albumin, pH 7·5, centrifuged and inoculated intracerebrally into infant mice. The virus strain was identified by complement-fixation test using sucrose–acetone extracted infected mouse brain as antigen and antisera from mice given six weekly intraperitoneal inoculations of mouse brain virus. Serum surveys were carried out by neutralization test, with sera undiluted or diluted to 1 : 4, heated at 56°C for 30 min and incubated for 1 h at 37°C with virus diluted to give 100 LD_{50} per mouse in final dose. The NT14952 strain was used after three or four passages in infant mouse brain.

Antisera for plaque-reduction tests were prepared in rabbits given six weekly intravenous inoculations of mouse brain virus. The sera were diluted in Hanks' solution with 0·014 M HEPES and 0·01 M sodium bicarbonate and heated at 56°C for 30 min. Virus–serum mixtures were incubated at 37°C for 1 h and assayed on PS–EK cells (Gorman *et al.* 1975).

Experimental inoculation of *Aedes aegypti* followed the techniques described by Carley *et al.* (1973).

Virus particles released from infected PS–EK cells were stained with 3% phosphotungstic acid, pH 6, for electron microscopy, by methods described previously (Lecatsas and Gorman 1972). The preparations were examined in a JEOL-100 C electron microscope (JEOL Limited, Tokyo, Japan) at an accelerating voltage of 80 kV and an instrumental magnification of 80 000.

Results

The number and species of arthropods submitted for virus isolation are listed in Table 1. *Culicoides marksi* and *Phlebotomus* sp. were considerably more abundant in the dry season than in the wet. This is consistent with findings at Charleville, Kowanyama and Beatrice Hill (Dyce, Muller, Standfast and Kay, unpublished data) which indicate that these insects are most abundant towards the end of the dry season. *C. marksi* readily attacks cattle and buffalo and has been shown to feed to a significant degree on the wallabies (*Wallabia agilis*) abundant in the area (Muller, Dyce, Standfast and Murray, unpublished data).

One virus strain, NT14952, was isolated from a pool of 118 *Culicoides marksi* collected by truck trap at Mudjinbarry on the evening of 19 September 1971. Reisolation from the original suspension was attempted unsuccessfully. Sucrose–acetone extracts of infected mouse brain did not agglutinate gander cells, but fixed complement with antiserum to Wallal virus but not with antisera to 32 other viruses isolated in Australia (Doherty 1977) or New Guinea (Woodroofe and Marshall 1971). Antiserum was prepared to the NT14952 strain and it and the prototype Ch12048 strain of Wallal virus were compared by neutralization and complement-fixation tests (Table 2). The strains were clearly distinguishable by neutralization test but not by complement-fixation. Similar antigenic differences were demonstrated, by plaque-reduction test (Table 3); NT14952 was clearly distinct from all seven available strains of Wallal virus. The antigenic differences are believed to justify recognition of NT14952 as a virus type in its own right, and the name 'Mudjinbarry' is suggested for it. Its resistance to exposure to sodium deoxycholate, the failure of 5-iodo-2-deoxyuridine to inhibit replication, its size by filtration (Table 4) and electron microscopy (Fig. 1) identified it as an orbivirus. The virus was shown to multiply after intrathoracic inoculation of *Aedes aegypti:* mosquitoes inoculated with $10^{1.6}$ LD_{50} per mosquito contained $10^{3.8}$–$10^{4.9}$ LD_{50} per mosquito 7–20 days later.

Neutralizing antibody to Mudjinbarry virus was found in a limited study in wallabies, but only equivocal reactions were shown in man, cattle or domestic fowls (Table 5). Five reactive wallaby sera gave either higher titres to Wallal virus or comparable titres to both viruses.

Discussion

The present findings increase the number of arboviruses reported in Australia to 35 and include the first isolation of a virus strain from insects collected in the Northern Territory, where antibody surveys (reviewed by Doherty 1974) have for some time suggested a high rate of arbovirus activity. It is proposed that Wallal and Mudjinbarry viruses be viewed as members of a Wallal antigenic group, known so far only from Australia. Further isolations of Wallal group viruses are needed, however, to exclude the possibility that strains may exist with a range of antigenic reactivity spanning the differences between Wallal and Mudjinbarry.

Table 1. Mosquitoes, midges and sandflies collected 24 March–14 April (wet season) and 15–27 September (dry season) 1971, and processed for virus isolation

Species	Wet season	Dry season
Psychodidae		
Phlebotomus sp.	0	997
Culicidae		
Anopheles (*Anopheles*) *bancroftii*	31	10
Anopheles (*Cellia*) *amictus*	216	21
Anopheles (*Cellia*) *annulipes*	171	44
Anopheles (*Cellia*) *farauti*	33	7
Anopheles (*Cellia*) *meraukensis*	341	42
Anopheles (*Cellia*) *novaguinensis*	15	2
Mimomyia (*Etorleptiomyia*) *elegans*	10	0
Coquillettidia (*Coquillettidia*) *xanthogaster*	7	0
Mansonia (*Mansonioides*) *uniformis*	5	0
Uranotaenia albescens	9	0
Aedes (*Ochlerotatus*) *normanensis*	118	20
Aedes (*Ochlerotatus*) *vigilax*	22	8
Aedes (*Neomelaniconion*) *lineatopennis*	24	17
Aedes (*Verrallina*) *carmenti*	26	0
Culex (*Culiciomyia*) *pullus*	594	0
Culex (*Culex*) *annulirostris*	1884	625
Culex (*Culex*) *bitaeniorhychus*	54	6
Culex (*Culex*) *fatigans*	244	25
Culex (*Culex*) *sitiens*	79	0
Culex (*Culex*) *starckeae*	6	0
Ceratopogonidae		
Lasiohelea sp.	344	19
Culicoides austropalpalis	136	243
Culicoides (*Avaritia*) spp.[A]	455	37
'*Culicoides bundyensis*'[B]	56	1
Culicoides bunrooensis	4	0
Culicoides dycei	19	6
Culicoides gemellus–Culicoides peregrinus	13	2
Culicoides marksi	440	2643
Culicoides sp. nr *nattaiensis*	1	1
Culicoides pallidothorax	14	0
Culicoides shultzei	104	1
Culicoides sp. mixed pool	14	0
Simuliidae		
Simulium sp.	40	0
Tabanidae		
Tabanus pallipennis	65	0
Tabanus obscurilineatus	40	0
Tabanus sp.	0	175
Muscidae		
Stomoxys calcitrans	50	0
Haematobia exigua	1000	0

[A] *Culicoides brevitarsis* dominant, *Culicoides actoni* secondary and two other undetermined *Culicoides* (*Avaritia*) spp. rare in these collections, but not differentiated in midges sorted for virus isolation.

[B] *Culicoides bundyensis* species complex.

Table 2. Antigenic comparison of Mudjinbarry and Wallal viruses

Antigens or viruses against which sera tested	Mouse antiserum to Mudjinbarry virus		Mouse antiserum to Wallal virus	
	Complement fixation titre[A]	Neutralization index[B]	Complement fixation titre[A]	Neutralization index[B]
Mudjinbarry (NT14952)	⩾ 64/8	2·5	64/8	0·8
Wallal (Ch12048)	⩾ 64/8	1·5	64/8	2·0

[A] Reciprocal serum titre/reciprocal antigen titre.
[B] Log_{10} neutralization index, determined by intracerebral inoculation of infant mice.

Table 3. Plaque-reduction tests of antisera prepared against Wallal virus strains to Wallal and Mudjinbarry viruses

Virus strain	Neutralization index[A] of antisera to:		Virus strain	Neutralization index[A] of antisera to:	
	Wallal	Mudjinbarry		Wallal	Mudjinbarry
Wallal			Wallal		
Ch12048	2·1	1·6	Ch12077	3·0	1·6
Ch11963	3·6	1·2	Ch12078	2·9	1·3
Ch11967	>3·6	1·8	Mudjinbarry		
Ch11999	2·8	1·3	NT14952	2·1	3·2
Ch12076	4·1	2·0			

[A] Log_{10} neutralization index, determined by plaque assay on PS–EK cells.

Table 4. Characterization of NT14952 (Mudjinbarry virus)[A]

Titre of mouse brain pool (3rd passage level)	
In mice	
Intracerebral inoculation of infant mice	6·8
Intraperitoneal inoculation of infant mice	<3·5
Intracerebral inoculation of weaned mice	<3·5
In cell culture	
Plaque assay on PS–EK cells	6·0
Sucrose–acetone extract of infected mouse brain	
No haemagglutination of gander cells at pH 6·0–7·6	
Fixation of complement[B]	64/16
Sensitivity to sodium deoxycholate[C]	4·4/4·3
Titre after filtration through membranes of average pore diameter:	
300 nm	4·5
220 nm	3·7
100 nm	<1·3
50 nm	<1·3
Sensitivity to 5-iodo-2-deoxyuridine[D]	7·0/7·0

[A] Titres as log_{10} LD_{50} (inoculation of mice) or log_{10} plaque forming units per ml on PS–EK cells (all other studies).
[B] Reciprocal serum titre/reciprocal antigen titre for homologous system.
[C] Technique of Sunaga *et al.* (1960): titre after incubation with 1 : 500 (w/v) sodium deoxycholate/titre after control incubation.
[D] Titre after assay in presence of 20 μg/ml of analogue/titre in control. Equine rhinopneumonitis virus, a known DNA virus, was inhibited in parallel assay and its inhibition was reversed by thymidine, confirming validity of the test.

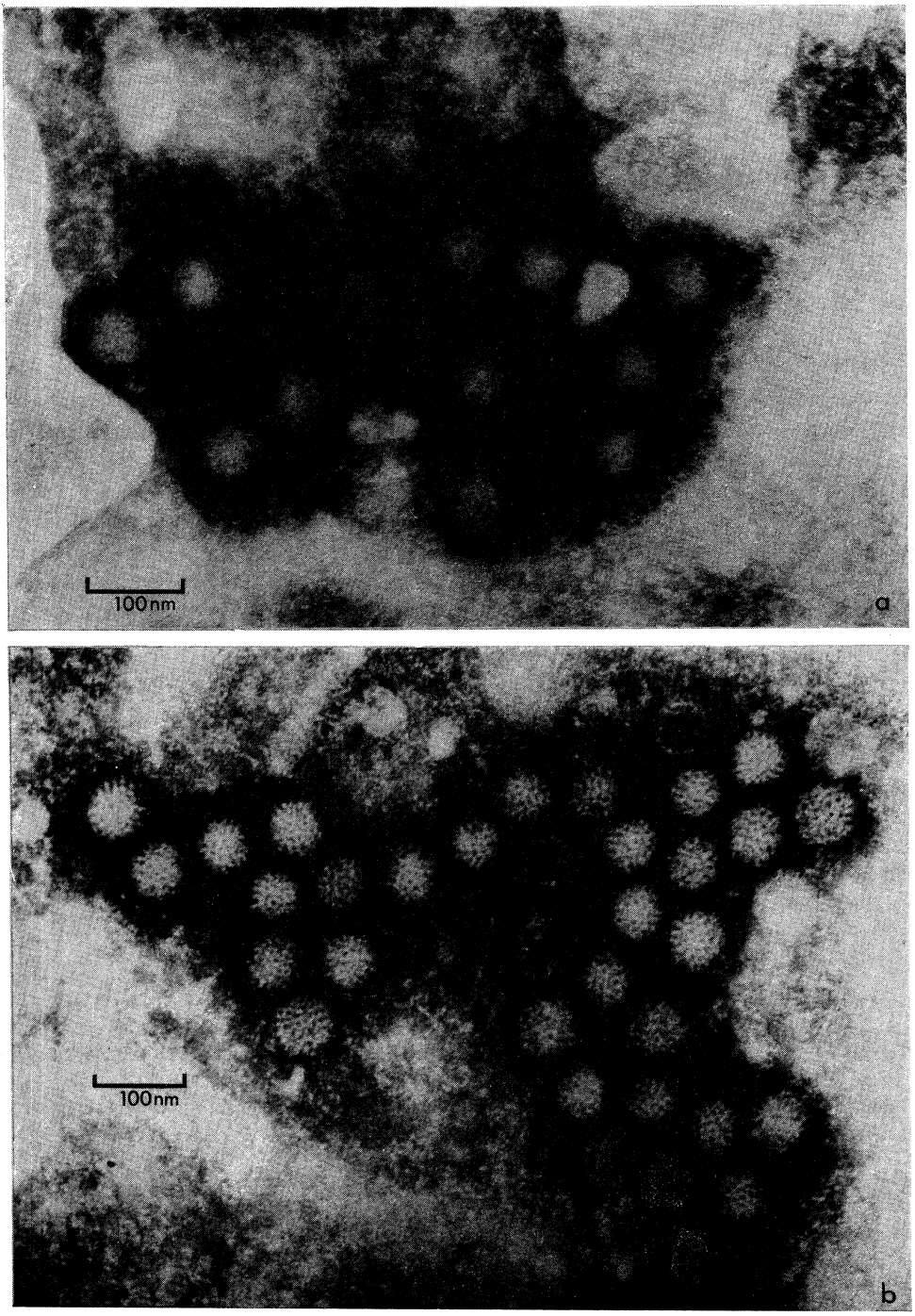

Fig. 1. Electron micrographs of Mudjinbarry virus stained with 3% phosphotungstic acid, pH 6·0. Diameter of virion (*a*) 69 nm and of nucleocapsid (*b*) 60 nm.

Table 5. Survey of neutralizing antibody to Mudjinbarry virus

Species	Place	Date	Number reactive/ number tested
Wallaby	Kowanyama	1967–68	5/30
Man	Victoria	1974	0/13
	Western Australia[A]	1974	1/25
	Central Australia[A]	1974	0/17
Cattle	Kowanyama	1969	0/30
Domestic fowl	Northern Territory	1974	1/30
Dingo	Central Australia	1974	2/12

[A] Sera from Aborigines.

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