Virus Name: Barmah Forest	Abbreviation: BFV

Status
Possible Arbovirus

Select Agent
No

SALS Level
2

SALS Basis
Placed at this biosafety level based on close antigenic or genetic relationship to other viruses in a group of 3 or more viruses, all of which are classified at this level.

Other Information

Antigenic Group
A7

SECTION I - Full Virus Name and Prototype Number

Prototype Strain Number / Designation
BH2193

Accession Number

Original Date Submitted
9/8/1984

Family
Togaviridae

Genus
Alphavirus

Information From
I.D. Marshall and G.M. Woodroofe; revised

Address
Microbiology Dept. JCSMR, Australian National University, Canberra, Australia, 2601

Information Footnote
Revised

Section II - Original Source

Isolated By (name)
I.D. Marshall and G.M. Woodroofe

Isolated at Institute
Canberra

Host Genus
Culex annulirostris

Species

Host Age/Stage
Adult

Sex
Female

Isolated From

Isolation Details

Signs and Symptoms of Illness

Arthropod
Depleted

Time Held Alive before Inoculation
Nil

Collection Method
CDC-type light trap

Collection Date
2/11/1974

Place Collected (Minimum of City, State, Country)
Barmah Forest, Victoria, Australia

Latitude
35° 50' S

Longitude
144° 12' E

Macrohabitat
River Red Gum Forest, Murray River flood plain

Microhabitat
Sandhills, grassed, partly flooded flats in forest

Method of Storage until Inoculated
Liquid nitrogen, Revco at -70dC

Footnotes

Section III - Method of Isolation

Inoculation Date
7/28/1976

Animal (Details will be in Section 6)
nb mice

Route Inoculated
ic-sc

Reisolation
Yes

Other Reasons
Unlike any virus held in this laboratory

Homologous Antibody Formation by Source Animal

Test(s) Used

Footnotes

Section IV - Virus Properties

Physicochemical
RNA, Single Strand

Pieces (number of genome segments)	Infectivity	Sedimentation Coefficients(s) (S)
Percentage wt, of Virion Protein	Lipid	Carbohydrate
Virion Polypeptides: Number **3 (1)**	Details **Core protein; 29,000 mw; glycoproteins (E1,E2): 56,000 mw, 43,000 mw (1)**	
Non-virion Polypeptides: Number	Details	
Virion Density	Sedimentation Coefficients(s) (S)	
Nucleocapsid Density	Sedimentation Coefficients(s) (S)	

Stability of Infectivity (effects)

pH (infective range)

Lipid Solvent (ether - % used to test)	After Treatment Titer	Control Titer
Lipid Solvent (chloroform)	After Treatment Titer	Control Titer
Lipid Solvent (deoxycholate) **1:200**	After Treatment Titer **<2.3 dex PFU/ml**	Control Titer **5.6 dex PFU/ml**

Other (formalin, radiation)

Virion Morphology

Shape	Dimensions **49 + 5 nm (1)**	
Mean nm	Range nm	
Measurement Method	Surface Projections/Envelope	Nucleocapsid Dimensions, Symmetry

Morphogenesis

Site of Constituent Formation in Cell	Site of Virion Assembly	Site of Virion Accumulation
Inclusion Bodies	Other	

Hemagglutination

Hemaggiutination	Antigen Source	Erythrocytes (species used)
Yes	**HA from Vero cells (1,2)**	**Gander**
pH Range	pH Optimum	
5.8-6.2	**6.0**	
Temperature Range	Temperature Optimum	
Romm temp, 37dC	**37dC**	

Remarks

Serologic Methods Recommended
CF, NT, HI

Footnotes

Section V - Antigenic Relationship and Lack of Relationship to Other Viruses

Initially, CF tests carried out at YARU by GMW showed relationship with Turlock group. Cross tests indicated identity with Umbre virus, but subsequently with higher titered immune ascitic fluid at JCSMR, identity was not confirmed. Cross neutralization tests at JCSMR indicated that BH2193/p2 is readily distinguish- able from Umbre prototype IG 1424/R620/p5.
Subsequently, plaque-purified Barmah Forest virus was shown to be related to alphaviruses by the HI test only. Barmah Forest virus did not react with any of the alphaviruses by CF or NT [1] , [2] . Interestingly, Umbre HA was inhibited in the HI test by antibody to Barmah Forest virus; and Umbre virus reacted ""weakly"" in the CF test with Barmah Forest virus. However, data obtained with regard to the mode of replication, morphology and morphogenesis, structural components of the virus and sequence homologies between the proteins of Barmah Forest virus and other alphaviruses have permitted the conclusion that Barmah Forest virus is an alphavirus with some apparently unusual features [1] .
In a separate study, BF virus was tested against 18 additional or a total of 24 alphaviruses by HI, CF and NT. Cross-reactions were not detected by CF and NT. Low level (titers of 10-80) cross-reactions were observed in the HI test, and with one exception, only when BF antigen reacted with antibodies to several of the various alphaviruses. In one instance, SF antigen was inhibited by BF antibody to a titer of 10 [2] .

Hemagglutination-Inhibition Tests with Barmah Forest Virus [1]

Immune Ascitic Fluid	Hemagglutinin					
	BFV	SFV	SIN	RRV	BEB	UMB
BFV	640 [a]	- [b]	-	-	-	-
Murweh	320					
Semliki Forest	20	240				
Sindbis	80	30	960			-
Whataroa	30					
Ross River	10	15		80		-
Getah	-					
Bebaru	20				60	-
Umbre	20	-	-	-	-	10240
Turlock	-					
Normal	-	-	-	-	-	-

[a] Reciprocal of dilution endpoint.

[b] No reaction at 1:10 dilution of immune ascitic fluid.

Section VI - Biologic Characteristics

Virus Source (all VERTEBRATE isolates)

Lab Methods of Virus Recovery (ALL ISOLATIONS)
Newborn mice; plaques on Vero cells; C6/36 and BHK-21 cell cultures

Cell system (a)	Virus passage history (b)	Evidence of Infection							
		CPE			PLAQUES			Growth Without CPE	
		Day (c)	Extent (d)	Titer TCD50/ml (e)	Day (c)	Size (f)	Titer PFU/ml (e)	+/- (g)	
Vero (CL)	SMB 2				5	5 mm	5.8*		
BHK-21 (CL)			CPE (5)						
C6/36 (CL)			3+-4+ (2)						

* Expressed in dex; In parallel tests Umbre did not form plaques

Section VII - Natural Host Range (Additional text can be added below table)

Vertebrate (species and organ) and arthropod	No. isolations/No. tested	No. with antibody/No. tested Test used	Country and region
Culex annulirostris	1/13,193		"Murray Valley", Victoria, Australia
Birds (mainly water birds)		1/378* PRNT	Hay, New South Wales Australia
Culex annulirostris	2/10,972		Charleville, S. Queensland, AS(1974)
Aedes normanensis	2/8,847		Australia (1976). "Murweh" virus (4)
Aedes bancroftianus	1/2,928		Barmah Forest, Murray Valley, AS (1981)
Aedes vigilax	2/1,300		Mogo Forest, Bateman's Bay, south coast N.S.W., AS(1984) (5)
Mixed pool:	1/1,300		Mogo Forest, Bateman's Bay, south coast N.S.W., AS
Coquillettidia linealis	(1984) (5)		
Culex molestus			
Culex cylindricus			
Man		9/300 NT	New South Wales (south coast), Australia (7)

* Positive from white-faced heron.

Antibodies to Barmah Forest virus have been found to be widespread in humans in New South Wales. Antibody studies showed a higher prevelence in the north coastal zones of the State and rates were significantly higher in male than in female subjects. (8)

Section VIII - Susceptibility to Experimental Infection (include viremia)

Experimental host and age	Passage history and strain	Inoculation Route-Dose	Evidence of infection	AST (days)	Titer log10/ml
Mice (nb)	SMB 5	ic 0.02	Hind leg paralysis, death	8.5	5.2 6.1 **
Mice (nb)		ip 0.03	Hind leg paralysis, death	9.5	3.8 4.2
Mice (nb)		sc	Hind leg paralysis, death		
Mice (wn)	SMB 2	ic 0.02	Antibodies		
Mice (wn)		ip 0.03	Antibodies		
			(Some paralyzed mice recover after all routes of inoculation)		

** First value = LD50; 2nd = ID50

Section IX - Experimental Arthropod Infection and Transmission

Arthropod species & virus source(a)	Method of Infection log10/ml (b)		Incubation period (c)		Transmision by bite (d)		Assay of arthropod, log10/ml (e)		
	Feeding	Injected	Days	°C	Host	Ratio	Whole	Organ	System
Aedes aegypti; infant mice 2 days after ic inoculation	7.0		0	27	N.D.	4.0-4.7		Vero PFU	
			7	Infant	6/10 (a)	4.5-6.4			
			21	mice	7/10	1.5-3.5			
			28		4/10	2.0-3.3			
			35		0/10	2.2-4.1			
			42		0/10	2.3-3.6			

(a) No all mosquitoes fed on any one day, but some transmitted without engorging. No mosquitoes fed or transmitted on days 35 and 42.

Section X - Histopathology

Character of lesions (specify host)

Inclusion Bodies — Intranuclear

Organs/Tissues Affected

Category of tropism

Section XI - Human Disease

In Nature — Residual — Death

Subclinical — Overt Disease

Clinical Manifestations

Number of Cases
*** See Remarks**

Category (i.e. febrile illness, etc.)

Section XII - Geographic Distribution

Known (Virus detected)
Murray Valley; N. Territory; S. Queensland; S. coast New South Wales, Australia

Suspected (Antibody only detected)

Section XIII - References

1. Dalgarno, L. et al. 1984. Virology 133:416-426.
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7. Vale, T.G., et al. 1985. Communicable Dis. Intelligence. 85/7:7-8.
8. Hawkes, R.A. et al. 1987. Med. J. Australia. 146:569-573.
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Remarks

*** A published report documents three patients in whom Barmah Forest viral infection appears to have resulted in illness (9).**