Virus Name: Royal Farm		Abbreviation: RFV
Status **Possible Arbovirus**	Select Agent **No**	SALS Level **2**

SALS Basis
Results of SALS surveys and information from the Catalogue.

Other Information

Antigenic Group
B

SECTION I - Full Virus Name and Prototype Number

Prototype Strain Number / Designation
ArT 371

Accession Number

Original Date Submitted
2/8/1985

Family
Flaviviridae

Genus
Flavivirus

Information From
Robert E. Williams

Address
U.S. Naval Medical Research Unit No. 3, Cairo, Egypt

Information Footnote
Reviewed by editor

Section II - Original Source

Isolated By (name)
U.S. NAMRU-3 (1)

Isolated at Institute
Cairo, Egypt

Host Genus
Argas (A.) hermanni

Species

Host Age/Stage
Nymphs

Sex
Not Answered

Isolated From

Isolation Details

Signs and Symptoms of Illness

Arthropod

Time Held Alive before Inoculation

Collection Method
By hand from pigeon farm

Collection Date
10/2/1968

Place Collected (Minimum of City, State, Country)
Royal Farm near Kabul, Afghanistan

Latitude

Longitude

Macrohabitat
Pigeon houses, and under seat covers and behind pictures tacked to walls in pigeon bazaars

Microhabitat

Method of Storage until Inoculated
Live ticks inoculated upon receipt at NAMRU-3

Footnotes

Section III - Method of Isolation

Inoculation Date
10/18/1968

Animal (Details will be in Section 6)
nb mice

Route Inoculated
Intracerebral

Reisolation
Yes

Other Reasons
No similar virus in laboratory.

Homologous Antibody Formation by Source Animal

Test(s) Used

Footnotes

Section IV - Virus Properties

Physicochemical

Pieces (number of genome segments)	Infectivity	Sedimentation Coefficients(s) (S)
Percentage wt, of Virion Protein	Lipid	Carbohydrate
Virion Polypeptides: Number	Details	
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) **Not done**	After Treatment Titer	Control Titer
Lipid Solvent (chloroform)	After Treatment Titer	Control Titer
Lipid Solvent (deoxycholate) **1:200**	After Treatment Titer **<1.0 dex**	Control Titer **>5.0 dex**

Other (formalin, radiation)

Virion Morphology

Shape	Dimensions	
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	**SMB ext. by sucrose-acetone**	**Goose**
pH Range	pH Optimum	
6.0-7.0	**6.4**	
Temperature Range	Temperature Optimum	
37dC		

Remarks

Serologic Methods Recommended
CF, HI, NT, agar gel diffusion

Footnotes

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

Relationship of Royal Farm virus by CF using immune serum to Royal Farm virus against antigens of 40-odd members of group B at NAMRU-3, YARU, and the Pasteur Institut, Dakar, gave titers of <16 with the exception of Langat and Powassan as shown in the table below.

HI and CF Tests

	Royal Farm Antigen					Royal Farm Immune Serum (2)			
	HI		CF			HI		CF	
Immune Sera	Ht/Ho	Ratio	Ht/Ho	Ratio	Antigens	Ht/Ho	Ratio	Ht/Ho	Ratio
Langat (3)	40/160	1/4	16/64	1/4	Langat	20/160	1/8	16/64	1/4
Powassan (6)	20/40	1/2	64/64	1	Powassan	10/40	1/4	I6/64	1/4

( ): Number of ip injections administered to mice

HI: serum diluted 1:5; 4 units of antigen used.

Neutralization Tests

	Royal Farm Virus [a]				Royal Farm Serum [b]		
	Undiluted	1:10	1:16		Undiluted	1:10	1:16
Immune Serum	Ht/Ho	Ht/Ho	Ht/Ho	Virus(a)	Ht/Ho	Ht/Ho	Ht/Ho
Langat (3) [b]	1.5/4.3	3.0/5.5	2.3/4.0	Langat	6.0/1.5	7.3/3.3	5.0/2.3
Powassan (3)			4.0/6.3	Powassan			9.3/2.3

[a] Freshly prepared virus in its 10th mouse brain passage was used.

[b] Sera used in the neutralization tests were produced by one formalized and 2 live injections at 14-day intervals; 5 injection Langat serum was used in the 1:16 dilution neutralization test; neutralization test results as LNI in dex.

Section VI - Biologic Characteristics

Virus Source (all VERTEBRATE isolates)

Lab Methods of Virus Recovery (ALL ISOLATIONS)
Newborn mice and chick embryos

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)	
HeLa (CL)	P-11	3-4	CPE						
Duck embryo (PC)		3	CPE						
Vero (CL)						No plaques(2)			
LLC-MK2 (CL)						No plaques(2)			
PS (CL)						No plaques(2)			
Duck embryo (PC)					6	Plaques	8.0* (2)		

* Expressed in dex

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
Argas (A.) hermanni	1/283		Kabul, Afghanistan
Argas (A.) hermanni	1/53		Kabul Province, Afghanistan

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)	P-2	ic .01	Paralysis, death	6	7.9
Mice (nb)	P-10	ip .02	Paralysis, death	9-12	11.2
Mice (nb)		sc			
Mice (wn)	P-2	ic .02	Paralysis, death	9	
Mice (wn)		ip .3	Antibody		
Mice (nb)	P-10	sc .02	Paralysis, death	9-12	11.7
chicks (1 day)	P-11	ip .02	Antibody		
chicks (1 day)		im .05	Antibody		
chicks (1 day)		sc .02	Antibody		
hamsters (3-4 wk)	P-10	ic .05	Paralysis, death	7	
rabbits (5 wk)		ic .05	Antibody		
guinea pigs (3 wk)		ic .05	Negative		
embryonated eggs (7 day)		ys .2	Death	4-6	
"" (10 day)		al.c..2	Death	5-7	
"" (10 day)		am.s..2	Death	5-7	

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

Section X - Histopathology

Character of lesions (specify host)
21-day-old hamster: pronounced perivascular cuffing throughout the cerebrum and brain stem; vessels surrounded by a cuff of lymphocytes, lymphoreticular cells, and plasma cells. Cuffs were conspicously absent in cerebellum.

Inclusion Bodies — Intranuclear

Organs/Tissues Affected
Brain (LV), lungs (LV), blood vessels (LV)

Category of tropism
Neurotropic in mouse, hamster, and chick

Section XI - Human Disease

In Nature	Residual	Death
Subclinical	Overt Disease	
Clinical Manifestations		
Number of Cases	Category (i.e. febrile illness, etc.)	

Section XII - Geographic Distribution

Known (Virus detected)
Afghanistan

Suspected (Antibody only detected)

Section XIII - References

1. Williams, R.E., et al. 1972. Am. J. Trop. Med. Hyg.. 21:582-586.
2. Calisher, C.H., et al. Personal communication. 1983.

Remarks