

Virus Name: Royal Farm

Abbreviation: RFV

Status: Possible Arbovirus
SALS Level: 2
Antigenic Group: B
Taxonomic status: *Flavivirus*
Other Information: None.

Select Agent:
SALS Basis: S

HEPA Filtration:

Section I - Full Virus Name and Prototype Number

Full Virus Name: Royal Farm
Prototype Number: ArT 371
Information from: Robert E. Williams *
Date: 2/8/1985
Address: U.S. Naval Medical Research Unit No. 3, Cairo, Egypt
*
Reviewed by editor

Section II - Original Source

Isolated by: U.S. NAMRU-3 (1) **at:** Cairo, Egypt
Genus and species: Argas (A.) hermanni **Sentinel** X
Age/Stage: Nymphs **Sex:**

Isolated From	Isolation detail
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Signs and symptoms of illness:
Arthropod engorged: depleted gravid
Time held alive before inoculation:
Collection date: 10/2/1968 **Method:** By hand from pigeon farm
Place collected: 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 and Validity

Inoculation Date: 10/18/1968
Animal: nb mice **Embryonated egg:** **Tissue Culture:**
(Details in Section VI - Biologic Char.)
Route inoculated: Intracerebral **Reisolation:** Yes
Other reasons: No similar virus in laboratory.
Homologous antibody formation by source animal (See Section II):
Test used: HI CF NT
Other:
Footnotes:

Section IV - Virus Properties

Physicochemical:

RNA: DNA: Single Strand: Double Strand:
Pieces: Infectivity: Sedimentation coefficient(s): /strong>
Percentage wt. of virion protein , lipid carbohydrate
Virion polypeptides:
Number: Details:
Non-virion polypeptides:
Number: Details:
Virion density: Sedimentation coefficient:
Nucleocapsid density Sedimentation coefficient:

Stability of infectivity (effects) pH

Lipid solvent:

(ether) Not done After treatment titer Control titer
(chloroform) After treatment titer Control titer

Detergent:

(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) how measured
Surface projections, envelope
Nucleocapsid dimensions, symmetry

Morphogenesis:

Site of constituent formation in cell

Site of virion assembly

Inclusion bodies

Other

Hemagglutination:

Hemagglutination Yes Antigen source SMB ext. by sucrose-acetone

Erythrocytes Goose pH range 6.0-7.0 pH optimum 6.4

Temperature optimum range 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									
Immune Sera	Royal Farm Antigen				Antigens	Royal Farm Immune Serum (2)			
	HI		CF			HI		CF	
	Ht/Ho	Ratio	Ht/Ho	Ratio		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	16/64	1/4

(): Number of ip injections administered to mice
 HI: serum diluted 1:5; 4 units of antigen used.

Neutralization Tests							
Immune Serum	Royal Farm Virus ^a			Virus(a)	Royal Farm Serum ^b		
	Undiluted	1:10	1:16		Undiluted	1:10	1:16
	Ht/Ho	Ht/Ho	Ht/Ho		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

Susceptibility of Cell Culture Systems:

Cell system (a)	Virus passage history (b)	Evidence of Infection						
		CPE			PLAQUES			Growth Without CPE +/- (g)
		Day (c)	Extent (d)	Titer TCD50/ml (e)	Day (c)	Size (f)	Titer PFU/ml (e)	
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

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 (Record Viremia)

Experimental host and age	Passage history and strain	Inoculation Route- Dose	Evidence of infection	AST (days)	Titer log ₁₀ /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)		Transmission by bite (d)		Assay of arthropod, log10/ml (e)		
	Feeding	Injected	Days	°C	Host	Ratio	Whole	Organ	System

Section X - Histopathology

Character of lesions: 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 conspicuously absent in cerebellum.

Inclusion bodies:

Cytoplasmic:(M) (LV) **Intranuclear:** (M) (LV)

Organs-tissues affected: Brain (LV), lungs (LV), blood vessels (LV)

Category of tropism: Neurotropic in mouse, hamster, and chick

Section XI - Human Disease

Human disease: **In nature:** (S) (R)
Death: (S) (R)
Residua: (S) (R)
Laboratory infections: **Subclinical:** (S) (R)
Overt Disease: (S) (R)
Clinical manifestations:
Category: **No. of cases:**

Section XII - Geographic Distribution

Known (virus):

Afghanistan

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.

Section XIV - Remarks
