

Isolation of Tyuleny Virus from Ticks *Ixodes* (*Ceratixodes*) *putus* Pick.-Camb. 1878 Collected on Commodore Islands

By

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Summary

Virus strain LEIV-284 Ka was isolated from *Ixodes* (*Ceratixodes*) *putus* tick collected in August, 1970, from *Phalacrocorax pelagicus* Pallas (pelagic cormorants) on Commodore Islands. The strain was shown to be identical with strains of Tyuleny virus, a group B arbovirus, recovered in 1969 on Tyuleny Island, Sea of Okhotsk. On Commodore Islands, hemagglutination inhibiting antibodies for the virus were found in 6% of the residents, 22% of fur seals, 24% of *Uria aalge* (common murres), 23% of *Lunda cirrhata* (tufted puffins) and in 11% of the *Rissa tridactyla* (black legged Kittiwakes).

1. Introduction

Tyuleny virus was first isolated in the U.S.S.R. in August, 1969, from ticks *Ixodes* (*Ceratixodes*) *putus* Pickard-Cambridge 1878 collected in the nesting grounds of *Uria aalge* on Tyuleny island, Sea of Okhotsk (2—5). The same virus was isolated by CLIFFORD *et al.* (6) on the Oregonian coast of the U.S.A.

According to the programme of the national committee for the study of viruses ecologically associated with birds, tick collections were undertaken in summer 1970 on the Commodore islands, Kamchatka region. The present paper reports the data on the isolation of a strain of Tyuleny virus from *Ixodes* (*Ceratixodes*) *putus* ticks collected at a colony of seabirds on Commodore islands.

2. Materials and Methods

Ixodes (*Ceratixodes*) *putus* (Pick.-Camb. 1878) ticks were collected in August, 1970, from *Phalacrocorax pelagicus* Pallas (pelagic cormorants) on a rock adjacent to the Pacific coast of Bering island, Commodore islands, the tundra zone of the subarctic regions. On the rock there were nesting grounds of seabirds, among which *Uria aalge*

(common murres) predominated on Bering island. Serum samples were obtained from adult Russian and Aleutian residents, fur seals and from the following species of birds: *Uria aalge Pontoppidau* (common murres), *Lunda cirrhata Pallas* (tufted puffins), *Rissa tridactyla Linnaeus* (blacklegged Kittiwakes); *Rissa brevirostris Brush*, *Phalacrocorax pelagicus Pallas* (pelagic cormorants). Isolation of the virus from ticks was attempted in 2-day-old suckling mice.

Identification of the virus isolates was done by hemagglutination inhibition (HI), complement-fixation (CF) and neutralization tests using suckling mice and chick fibroblast tissue cultures. Antigens were prepared by the sucrose-acetone method (1). To remove inhibitors, sera of human beings and of fur seals were treated with kaolin, sera of birds were treated with acetone according to the standard techniques used everywhere.

Sera were tested with antigens prepared from the following group B arboviruses: Tyuleny, West Nile, Japanese encephalitis and tick-borne encephalitis.

3. Results

Strain LEIV-284 Ka was obtained from a pool of ticks *Ixodes (Ceraticoxodes) putus* collected from *Phalacrocorax pelagicus Pallas*. Reisolation of the strain was made from the primary material stored at -70°C for 1.5 months. The virus agglutinated goose red cells within an optimal pH range of 6.4 to 6.5.

Table 1. *Results of Identification of the Strain*

Antigens	Sera HIT ^a			CFT ^a			Sakha- lin	NT	
	Group A	Group B		Group A	Group B			Mice ^b Tyuleniy	TC ^c
		Group B	Tyule- niy		Group B	Tyule- niy			
LEIV-284 KA	0	40	160	0	20	80	0	2.0	1.9
Tyuleniy (—6c)	0	20	160	0	20	80	0	2.0	2.6
Sakhalin	—	—	—	0	0	0	80	—	—

^a reciprocals of antibody titers.

^b log NI in suckling mice.

^c log NI in chick fibroblast tissue culture.

The serological identification of the isolated strain indicated that it was identical with the prototype strain LEIV-6C of Tyuleny virus (Table 1). The cultural behaviour of strains 284 Ka and 6C of Tyuleny virus also proved to be similar. In chick fibroblast tissue cultures both strains caused cytopathic effects, formed plaques about 2 mm in diameter and produced hemagglutinins in a titer of 1 : 4 to 1 : 8 in the culture fluid 48 hours post infection. On the basis of these observations it was, therefore, concluded that strain 284 Ka belongs to Tyuleny virus.

The results of the HI test performed with sera of human beings, fur seals and birds are summarized in Table 2.

Antibodies were found in sera of two adult residents. High titers of antibodies were regularly demonstrated in fur seals, *Uria aalge* and *Lunda cirrhata Pallas*. The lack of antibodies in *Phalacrocorax pelagicus Pallas* appears to be due to the fact that the amount of the material tested was not representative. Three *Uria aalge* (9%) and one *Lunda cirrhata Pallas* (4%) showed high titers of antibodies

Table 2. *Hemagglutination Inhibiting Antibodies for Tyuleniy Virus in the Sera of Human Beings, Fur Seals and Birds Collected on Commodore Islands*

Hosts	Sera tested						
	Total No.	Positive		Antibody titres			
		No.	%	1:20	1:40	1:80	1:160
Residents	33	2	6	—	1	1	—
Fur seals	64	14	22	3	6	2	3
Birds:							
Common murre (<i>Uria aalge</i>)	33	8	24	4	4	—	—
Tufted Puffin (<i>Lunda cirrhata</i>)	26	6	23	2	3	1	—
Blacklegged Kittywake (<i>Rissa tridactyla</i>)	18	2	11	2	—	—	—
Redlegged Kittywake (<i>Rissa brevirostris</i>)	6	0	—	—	—	—	—
Pelagic cormorant (<i>Phalacrocorax pelagicus</i>)	5	0	—	—	—	—	—

(1 : 20, 1 : 40, 1 : 640) for Japanese encephalitis virus, however, they lacked antibodies to other representatives of group B arboviruses.

4. Discussion

The isolation of Tyuleniy virus on Commodore islands along with the isolation of the same virus (Three Arch virus) by CLIFFORD *et al.* (6) in the North of the Pacific coast in U.S.A., Oregon, have confirmed the prognoses made earlier (3—5). The predicted geographical distribution of the virus, probably coinciding with that of *I. putus* ticks needs a further investigation.

The results of a serological survey indicated possible infection of man with Tuleniy virus in fact. According to our observation, up to 50 ticks actively attack a human bait during 20 minutes exposure on the breeding territory of seabirds. Serological data also suggest that fur seals along with birds actively participate in the ecological circulation of the virus. The pathogenicity of the virus for man, fur seals and colonial birds, however remains questionable.

Addendum

The identity of strains of Tyuleniy virus originating from the U.S.S.R. and the U.S.A. (Three Arch virus) was established by Dr. Casals at the World Reference Center for Arboviruses at YARU in January, 1971, by complement-fixation and neutralization tests. In addition, results obtained by complement fixation tests indicated that Tyuleniy virus differed from the following group B arboviruses:

1. Four-fold difference of homologous and heterologous titers with Alfuy, Ilheus, Israel turkey meningoencephalitis, Kadam, tickborne encephalitis and yellow fever viruses.

2. Eight-fold difference of titers with Apoi, Banzi, Edge Hill, Japanese encephalitis, Langat, Murray Valley, Omsk hemorrhagic fever, Powassan, Tembusu, Usutu, Wesselsbron and West Nile viruses.

3. Sixteen-fold difference of titers with Bussuquara, Central European Tick-borne, dengue-4, Kunjin, Louping-ill, Negishi, Ntaya and Spondweni viruses.

4. Negative results ($<1:8$) were obtained with Cowbone Ridge, Dakar bat, dengue 1, dengue 2, dengue 3, Egypt Art, Entebbe bat, Ibadan, An 10069, Kokohera, MML, Modoc, Stratford and Zika viruses.

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