

IDENTITY OF KARELIAN FEVER AND OCKELBO VIRUSES DETERMINED BY SERUM DILUTION-PLAQUE REDUCTION NEUTRALIZATION TESTS AND OLIGONUCLEOTIDE MAPPING

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Abstract. The causative agents of Ockelbo disease in Sweden, Pogosta disease in Finland, and Karelian fever in the USSR have been attributed to alphaviruses (family *Togaviridae*) related to Sindbis virus. We compared prototypes Sindbis, Ockelbo, and Karelian fever viruses by neutralization tests. We also analyzed oligonucleotide fingerprint maps of prototypes Ockelbo and Karelian fever viruses and a strain of Sindbis virus from Czechoslovakia. The results indicate that Ockelbo and Karelian fever viruses are essentially identical and suggest that Ockelbo disease, Pogosta disease, and Karelian fever are synonyms for the same disease.

Karelian fever is an acute febrile illness in humans characterized by mild to severe (often persistent) polyarthritides and a rash.¹ The disease occurs in the far western Soviet Union near the border of Finland. The epidemiology of Karelian fever suggests that it is arthropod-borne, as the onset of illness coincides with rural exposure in seasons of highest arthropod population densities.

Similar illnesses, known as Pogosta disease and Ockelbo disease, have been reported in Finland and Sweden, respectively.²⁻⁴ Serum samples from patients with Karelian fever, Ockelbo disease, or Pogosta disease contain antibodies that react with Sindbis virus (family *Togaviridae*, genus *Alphavirus*).¹⁻⁵ A virus has not been isolated from humans with this illness; however, the probable etiologic agent has been isolated from mosquitoes of the *Culiseta* species collected in areas of endemic disease in Sweden⁶ and from mosquitoes of the *Aedes* (probably *communis*) species in the Karelian region of the western USSR.⁷ Although both these viruses have been compared antigenically with Sindbis virus,^{6,7} simultaneous antigenic comparisons of the 3 viruses have not been done.

This paper reports a comparison of prototypes

Sindbis, Ockelbo, and Karelian fever viruses by serum dilution-plaque reduction neutralization tests. The results indicate that Ockelbo and Karelian fever viruses are essentially identical and that both differ from but are related antigenically to Sindbis virus in essentially the same way. Further, analyses of oligonucleotide fingerprint maps indicate that Ockelbo and Karelian fever virus strains differ from each other by a single oligonucleotide.

MATERIALS AND METHODS

Virus and antibody preparations

All viruses except Karelian fever virus were from the reference collection of the Division of Vector-Borne Viral Diseases, Centers for Disease Control, Fort Collins. Prototype Karelian fever virus¹ was from the reference collection of the D. I. Ivanovsky Institute, Moscow. Virus strains used were as follows: for Sindbis virus, strain Michalovce, isolated from a hamster in Czechoslovakia,⁸ and prototype strain EgAr 339, isolated from *Culex univittatus* in Egypt;⁹ for prototype Ockelbo virus, strain Edsbyn 5/82; and for Karelian fever virus, strain LEIV-9298. Stock virus was prepared by passing seed virus in suckling (2-4-day-old) mice inoculated intracranially

TABLE 1

Prototypes Sindbis (EgAr 339), Ockelbo (Edsbyn 5/82), and Karelian fever (LEIV-9298) viruses compared by serum dilution-plaque reduction neutralization tests in Vero cells

Virus	Titer of neutralizing antibody		
	Sindbis	Ockelbo	Karelian fever
Sindbis	>1,280	160	80
Ockelbo	40	160	40
Karelian fever	80	160	80

(ic); when the mice showed signs of illness, they were frozen. Ten percent suspensions of brains from infected suckling mice were clarified by centrifugation and aliquots frozen at -70°C until used for titrations, neutralization tests, or for preparation of working virus to be used in oligonucleotide fingerprint analyses. Antibody was prepared as hyperimmune mouse ascitic fluids according to the method of Tikasingh and others.¹⁰

Neutralization tests

After titration by plaque assay in Vero cell cultures, prototypes Sindbis and Ockelbo and Karelian fever virus strain LEIV-9298 were cross-compared by serum dilution-plaque reduction neutralization tests using a single agar overlay containing neutral red. The method has been published.¹¹

RNase T1 oligonucleotide fingerprint analysis

Viruses were cultured in BHK-21 cells and purified from the supernatant fluids by polyethylene glycol precipitation and centrifugation in glycerol-potassium tartrate gradients.¹² Extraction of RNA from purified virions and end-labeling of the RNase T1-resistant oligonucleotides were done as previously described.¹³ Two dimensional polyacrylamide gel electrophoresis of ^{32}P labeled oligonucleotides was performed according to DeWachter and Fiers.¹⁴

RESULTS

Suckling mice inoculated with prototype viruses Sindbis or Ockelbo, or with Karelian fever virus strain LEIV-9298, died 2–3 days later. Pro-

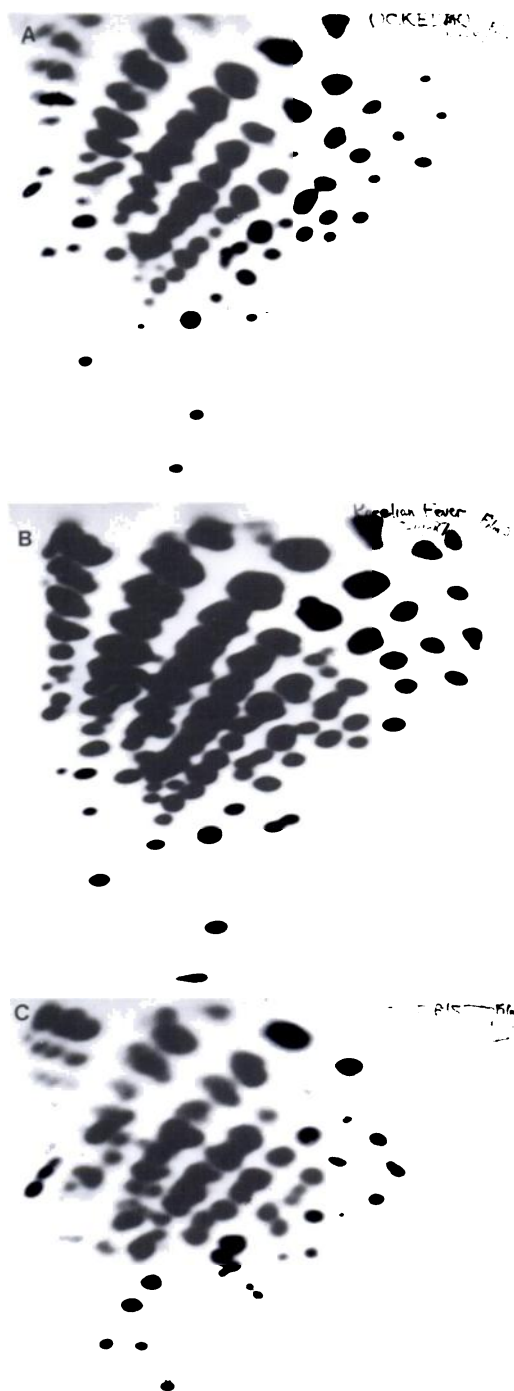


FIGURE 1. RNase T1 oligonucleotide fingerprints of the 42S RNA genomes of (A) Ockelbo, (B) Karelian fever, and (C) Sindbis viruses.

prototype Ockelbo and Karelian fever virus strain LEIV-9298 formed plaques 3–4 mm in diameter 3 days after inoculation and had titers of $10^{8.6}$ and $10^{8.4}$ plaque-forming units per ml in Vero cell cultures, respectively. Prototype Sindbis virus formed plaques 1.5–2 mm in diameter 3 days after inoculation and had titers of $10^{8.4}$ plaque-forming units per ml in Vero cell cultures.

By cross-neutralization testing (Table 1), both Ockelbo and Karelian fever virus strain LEIV-9298 are subtypes of the prototype Sindbis virus strain from Egypt. Furthermore, prototype Ockelbo and Karelian fever virus strain LEIV-9298 are indistinguishable.

The oligonucleotide fingerprints of Czechoslovakian strain of Sindbis virus, prototype Ockelbo virus, and Karelian fever virus strain LEIV-9298 were compared to determine the genetic relatedness of these alphaviruses (Fig. 1). Fingerprints of Ockelbo (Fig. 1A) and Karelian fever (Fig. 1B) viruses were essentially identical, although the map of Karelian fever virus shows 1 additional oligonucleotide. The fingerprint of the Sindbis virus isolate from Czechoslovakia (Fig. 1C) was distinct from those of Ockelbo or Karelian fever viruses. Comparisons of Ockelbo and Karelian fever virus oligonucleotide fingerprints with those of other Sindbis virus isolates¹⁵ or other alphaviruses¹⁶ indicated that Ockelbo and Karelian fever viruses are distinct, but so similar that they can be considered isolates of the same virus.

DISCUSSION

No virus has been isolated from a human with Ockelbo disease, Karelian fever, or Pogosta disease. However, prototypes Ockelbo virus and Karelian fever viruses were isolated under circumstances that would suggest they are candidates for such recognition. The similarity of these illnesses found in a region of northeastern Europe (Sweden, Finland, and western USSR), isolations of alphaviruses from mammal-feeding *Culiseta* and *Aedes* mosquitoes in conjunction with epidemics of the disease, and the virtual identity of prototype Ockelbo virus and Karelian fever virus strain LEIV-9298 suggest that Ockelbo and Karelian fever viruses are strains of the same virus and that this virus is the etiologic agent of Ockelbo disease in Sweden, Pogosta disease in Finland, and Karelian fever in the USSR.

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