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SINDBIS VIRUS AND POGOSTA DISEASE IN FINLAND

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Your theory is crazy, but it's not crazy enough to be true

- Niels Bohr (1885-1962)

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LIST OF ORIGINAL PUBLICATIONS

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I Tytti MANNI, Satu KURKELA, Antti VAHERI, Olli VAPALAHTI
Diagnostics of Pogosta disease: Antigenic properties and evaluation of Sindbis virus IgM and IgG enzyme immunoassays
Vector-Borne and Zoonotic Diseases, in press

II Satu KURKELA, Tytti MANNI, Antti VAHERI, Olli VAPALAHTI
Causative agent of Pogosta disease isolated from blood and skin lesions
Emerging Infectious Diseases 2004;10:889-894

III Satu KURKELA, Tytti MANNI, Johanna MYLLYNEN, AnttiVAHERI, Olli VAPALAHTI
Clinical and laboratory manifestations of Sindbis virus infection: Prospective study, Finland, 2002-2003
The Journal of Infectious Diseases 2005;191:1820-1829

IV Satu KURKELA, Tapani HELVE, Antti VAHERI, Olli VAPALAHTI
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Sindbis virus infection in migratory birds, grouse, and humans, Finland
Emerging Infectious Diseases, in press

ABBREVIATIONS

arbovirus	arthropod-borne virus
BFV	Barmah Forest virus
BSA	bovine serum albumin
C	capsid protein
CHIKV	Chikungunya virus
cDNA	complementary deoxyribonucleic acid
CPE	cytopathic effect
CSF	cerebrospinal fluid
E1	envelope glycoprotein 1
E2	envelope glycoprotein 2
EDTA	ethylene diamine tetra-acetic acid
EEEV	eastern equine encephalitis virus
EIA	enzyme immunoassay
HI	hemagglutination inhibition
IFA	immunofluorescence assay
MAYV	Mayaro virus
MEM	minimal essential medium
NT	neutralization test
OD	optical density
ONNV	O'nyong-nyong virus
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
RRV	Ross River virus
RT-PCR	reverse transcriptase polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SINV	Sindbis virus
VEEV	Venezuelan equine encephalitis virus
WEEV	western equine encephalitis virus

ABSTRACT

Sindbis virus (SINV) (genus *Alphavirus*, family *Togaviridae*) is an enveloped virus with a genome of single-stranded, positive-polarity RNA of 11.7 kilobases. SINV is widespread in Eurasia, Africa, and Australia, but clinical infection only occurs in a few geographically restricted areas, mainly in Northern Europe. In Europe, antibodies to SINV were detected from patients with fever, rash, and arthritis for the first time in the early 1980s in Finland. It became evident that the causative agent of this syndrome, named Pogosta disease, was closely related to SINV. The disease is also found in Sweden (Ockelbo disease) and in Russia (Karelian fever). Since 1974, for unknown reason, the disease has occurred as large outbreaks every seven years in Finland. This study is to a large degree based on the material collected during the 2002 Pogosta disease outbreak in Finland.

We first developed SINV IgM and IgG enzyme immunoassays (EIA), based on highly purified SINV, to be used in serodiagnostics. The EIAs correlated well with the hemagglutination inhibition (HI) test, and all individuals showed neutralizing antibodies. The sensitivities of the IgM and IgG EIAs were 97.6% and 100%, and specificities 95.2% and 97.6%, respectively. E1 and E2 envelope glycoproteins of SINV were shown to be recognized by IgM and IgG in the immunoblot early in infection.

We isolated SINV from five patients with acute Pogosta disease; one virus strain was recovered from whole blood, and four other strains from skin lesions. The etiology of Pogosta disease was confirmed by these first Finnish SINV strains, also representing the first human SINV isolates from Europe. Phylogenetic analysis indicated that the Finnish SINV strains clustered with the strains previously isolated from mosquitoes in Sweden and Russia, and seemed to have a common ancestor with South-African strains. Northern European SINV strains could be maintained locally in disease-endemic regions, but the phylogenetic analysis also

suggests that redistribution of SINV tends to occur in a longitudinal direction, possibly with migratory birds.

We searched for SINV antibodies in resident grouse (N=621), whose population crashes have previously coincided with human SINV outbreaks, and in migratory birds (N=836). SINV HI antibodies were found for the first time in birds during their spring migration to Northern Europe, from three individuals: red-backed shrike, robin, and song thrush. Of the grouse, 27.4% were seropositive in 2003, one year after a human outbreak, but only 1.4% of the grouse were seropositive in 2004. Thus, grouse might contribute to the human epidemiology of SINV.

A total of 86 patients with verified SINV infection were recruited to the study in 2002. SINV RNA detection or virus isolation from blood and/or skin lesions was successful in eight patients. IgM antibodies became detectable within the first eight days of illness, and IgG within 11 days. The acute phase of Pogosta disease was characterized by arthritis, itching rash, fatigue, mild fever, headache, and muscle pain. Half of the patients reported in self-administered questionnaires joint symptoms to last > 12 months. Physical examination in 49 of these patients three years after infection revealed persistent joint manifestations. Arthritis (swelling and tenderness in physical examination) was diagnosed in 4.1% (2/49) of the patients. Tenderness in palpation or in movement of a joint was found in 14.3% of the patients in the rheumatologic examination, and additional 10.2% complained persisting arthralgia at the interview. Thus, 24.5% of the patients had joint manifestations attributable to the infection three years earlier. A positive IgM antibody response persisted in 3/49 of the patients; both two patients with arthritis were in this group. Persistent symptoms of SINV infection might have considerable public health implications in areas with high seroprevalence.

The age-standardized seroprevalence of SINV (1999-2003, N=2529) in the human population in Finland was 5.2%. The seroprevalence was high in North Karelia, Kainuu, and Central Ostrobothnia. The incidence was highest in North Karelia. Seroprevalence in men (6.0%) was significantly higher than in women (4.1%), however, the average annualized incidence in the non-epidemic years was higher in women than in men, possibly indicating that infected men are more frequently asymptomatic. The seroprevalence increased with age, reaching 15.4% in persons aged 60-69 years. The incidence was highest in persons aged 50-59 years.

TIIVISTELMÄ (SUMMARY IN FINNISH)

Sindbis-virus kuuluu Togavirusten heimoon ja alfavirusten sukuun, ja sen genomina on yksittäinen positiivisäikeinen n. 11,7 kiloemäksen pituinen RNA. Sindbis-virusta esiintyy Euraasiassa, Afrikassa ja Australiassa, mutta ihmisen oireisia infektioita lähinnä vain Pohjois-Euroopassa. Euroopassa Sindbis-virusvasta-aineita osoitettiin nivel-ihottuma-kuumeoireisilta potilailta ensi kerran 1980-luvun alussa, Suomessa. Vasta-ainetutkimukset osoittivat, että taudinaiheuttaja on läheistä sukua Sindbis-virukselle; tauti nimettiin pogostantaudiksi. Ruotsissa taudin nimi on Ockelbosjuka ja Venäjällä *karelskaja lihoradka*, ”karjalankuume”. Vuodesta 1974 lukien tauti on tuntemattomasta syystä puhjennut epidemiaksi seitsemän vuoden välein. Tämä tutkimus pohjautuu pitkälti vuoden 2002 pogostantautiepidemian aikana koottuun aineistoon.

Kehitimme laboratoriodiagnostiikkaan soveltuvat entsyymi-immunologiseen määritykseen (EIA) perustuvat vasta-ainetestit, joilla voidaan osoittaa Sindbis-virus-IgM- ja IgG-vasta-aineita seerumista. Menetelmien antigeenina on puhdistettu kokoviruspreparaatti. Testien osoitettiin korreloivan hyvin hemagglutinaation inhibitiotestin kanssa. Kaikilta testattavilta osoitettiin neutraloivia vasta-aineita. IgM-EIA:n sensitiivisyys oli 97.6% ja spesifisyys 95.2%. IgG-EIA:n sensitiivisyys oli 100% ja spesifisyys 97.6%. Infektion alkuvaiheen IgM- ja IgG-vasta-aineet tunnistivat E1- ja E2-glykoproteiinit immunoblottauksessa.

Eristimme Sindbis-viruksen viidestä akuutista pogostantautipotilaasta (yksi kokoverestä ja neljä iholeesioista), mikä varmisti Sindbis-viruksen pogostantaudin aiheuttajaksi. Nämä ovat ensimmäiset suomalaiset Sindbis-viruskannat, ja ensimmäiset ihmisestä eristetyt kannat Euroopassa. Fylogenia-analyysi osoitti suomalaisten kantojen olevan läheistä sukua Ruotsissa ja Venäjällä hyttysistä eristetyille kannoille sekä eteläafrikkalaisille kannoille. Pohjoiseurooppalaiset

kannat saattavat kiertää paikallisesti endeemisillä alueilla, mutta virus voi myös levitä pitkiä matkoja pohjois-eteläsuunnassa, mahdollisesti muuttolintujen avulla.

Metsäkanalintujen populaation on osoitettu romahtavan joka 6.-7. vuosi, muistuttaen pogostantaudin epidemiasykliä. Tutkimme 621 metsäkanalintua sekä 836 muuttolintua. Sindbis-virusvasta-aineita voitiin osoittaa ensi kertaa Pohjois-Eurooppaan saapuvista muuttolinnuista kevätmuuton aikana. Vasta-aineita osoitettiin kolmesta yksilöstä; pikkulepinkäisestä, punarinnasta ja laulurastaasta. Metsäkanalinnuista 27,4 % oli vasta-ainepositiivisia vuonna 2003 eli vuoden kuluttua pogostantautiepidemiasta. Vuonna 2004 ainoastaan 1,4 % oli positiivisia. Metsäkanalinnuilla voi siis olla rooli pogostantaudin erikoisessa epidemiologiassa.

86 serodiagnosoitua pogostantautipotilasta osallistui tutkimukseen. Sindbis-virus-RNA:n osoittaminen tai viruseristys kokoverestä tai iholeesiosta onnistui 8 potilaasta. IgM-vasta-aineita osoitettiin viimeistään 8. ja IgG-vasta-aineita 11. sairauspäivänä. Akuutit tyyppioireet olivat niveloireet, kutiava ihottuma, väsymys, lievä kuume, päänsärky ja lihaskipu. Puolet potilaista ilmoitti niveloireiden jatkuneen yli vuoden ajan. Tutkimme samat potilaat kolmen vuoden kuluttua infektiosta havaitaksemme pitkittyneitä nivelvaivoja objektiivisesti. Tässä vaiheessa 49 potilasta osallistui tutkimukseen. Artriitti (statuksessa turvotus ja arkuus) todettiin 4,1 %:lla (2/49) potilaista. Palpaatio- tai liikearkuus todettiin 14,3 %:lla statuksessa. Lisäksi 10,2 % ilmoitti haastattelussa pitkittynyttä nivelkipua. Kaikkiaan 24,5 %:lla oli niveloireita, jotka olivat ajallisesti liitettävissä pogostantaudin alkuun ja jotka eivät selittyneet muulla sairaudella. IgM-vasta-aineita osoitettiin 3/49 potilaista; molemmat artriitti-potilaat olivat IgM-positiivisia. Pohjois-Karjalassa noin 10 % väestöstä on sairastanut pogostantaudin; arvion mukaan maakunnan alueella on satoja potilaita, joiden krooninen niveltulehdus johtuu aiemmasta Sindbis-virusinfektiosta. Pogostantaudin mahdollisuus tulisi huomioida epäselvien niveltulehdusten erotusdiagnostiikassa.

Suomalaisten ikävakioitu Sindbis-viruksen seroprevalenssi (1999-2003, N=2529) oli 5,2 %. Seroprevalenssi oli korkea Pohjois-Karjalassa, Kainuussa ja Keski-Pohjanmaalla. Insidenssi oli korkein Pohjois-Karjalassa. Miesten seroprevalenssi (6,0 %) oli merkitsevästi korkeampi kuin naisten (4,1 %), mutta naisilla insidenssi oli miehiä korkeampi. Seroprevalenssi nousi iän mukana ja se oli korkein 60-69-vuotiailla (15,4 %). Insidenssi oli korkein 50-59-vuotiailla.

REVIEW OF THE LITERATURE

1. ARBOVIRUSES AS HUMAN PATHOGENS

Arboviruses or arthropod-borne viruses include several hundred viruses that are primarily transmitted by arthropods, e.g. mosquitoes, ticks, and sandflies; i.e. arthropods function as vectors for arboviruses. These viruses are maintained in a non-human vertebrate host, and transmission to humans or other vertebrates occurs through blood feeding by vectors. Some arboviruses are, in addition, maintained by a vertical transmission from the female adult to its offspring. Arboviruses replicate in both arthropod and vertebrate cells.

Arboviruses that are pathogenic to humans are a mixed collection from five different taxonomical families: *Bunyaviridae, Flaviviridae, Reoviridae, Rhabdoviridae*, and *Togaviridae* (**Table 1**). Many of them can cause a severe human disease, such as yellow fever, dengue, and Japanese encephalitis, which all are globally recognized infections. The clinical diseases caused by arboviruses vary from an encephalitic disease to hemorrhagic fever or rash-arthritis. The infection may also remain asymptomatic or subclinical. Humans are usually dead-end hosts for arboviruses, unable to produce significant viremia.

Human pathogenic arboviruses currently known to circulate in Finland are Inkoo virus (*Bunyaviridae*) [30], Sindbis virus (SINV) (*Togaviridae*) [28], and tick-borne encephalitis virus (*Flaviviridae*) [245]. Acute Inkoo virus infection is rarely serodiagnosed, but this is most probably due to low clinical alert, and subsequently considerable underdiagnosis [201]. A few tens of tick-borne encephalitis cases are serodiagnosed annually, mostly in the Åland islands [244]. The number of acute SINV infections serodiagnosed annually varies from only few cases to over a thousand [31], and thus, in average, SINV infection is the most commonly serodiagnosed arbovirus infection in Finland.

2. INTRODUCTION TO ALPHAVIRUSES

CLASSIFICATION, GENOMIC STRUCTURE, AND REPLICATION

The taxonomic virus family *Togaviridae* consists of the genus *Alphavirus* and the genus *Rubivirus*. The best known representative in the genus *Rubivirus* is rubella virus, the causative agent of German measles. In the literature, the genus *Alphavirus* has been divided into seven antigenic (sero)complexes: Barmah Forest, eastern equine encephalitis, Middelburg, Nduma, Semliki Forest, Venezuelan equine encephalitis, and western equine encephalitis [41].

The alphavirus virion is spherical, ~70 nm in diameter (**Figure 1**), and consists of a nucleocapsid enclosed within a lipoprotein envelope [238]. The nucleocapsid and envelope proteins are arranged in an icosahedral (T = 4) symmetry. Alphaviruses have a linear single-stranded positive-polarity genome of 11-12-kilobase RNA, with a 5′-terminal cap structure and 3′-poly(A) tail, and which serves as a messenger RNA in mammalian cells [227].

Alphaviruses attach to the host cell via the glycoprotein spikes of the viral surface and enter the cell by endocytosis in clathrin-coated pits [63]. In the low pH of endosomes, the E1/E2 heterodimers dissociate. E1 units trimerize and expose their fusion peptides, which drives the fusion of the endosomal and viral membranes [142]. During the exposure to the cytoplasm, the nucleocapsids disassemble. The infective genome is released to the cytoplasm. The 5′ two-thirds of the genomic RNA is translated to produce the polyproteins P123 and P1234, from which the non-structural proteins nsP1-4 are proteolytically cleaved [205]. The P1234 is also a precursor for the RNA polymerase. A subgenomic 26S messenger RNA, transcribed from the terminal one-third of the genomic RNA, encodes a structural polyprotein p130, from which the structural proteins C (capsid), 6K, and E1 (~50 kd), E2 (~45 kd), and E3 envelope glycoproteins are proteolytically cleaved [84,122,205] (**Figure 2**).

The C proteins interact with each other and with viral RNA in order to assemble into nucleocapsids. The viral membrane glycoproteins are translocated into the lumen of endoplasmic reticulum [95]. The E3/E2-precursor (p62) and E1 are cleaved cotranslationally in the endoplasmic reticulum by signal peptidase [143]. p62 is cleaved by furin to E2 and E3 during the exit from the trans-Golgi

Table 1.

Arboviruses with a known human pathogenicity. Viruses with yet unestablished pathogenicity (e.g. only one case report) are not included. The reference in which connection to human disease was first reported is indicated. M, mosquito; T, tick, S, sandfly

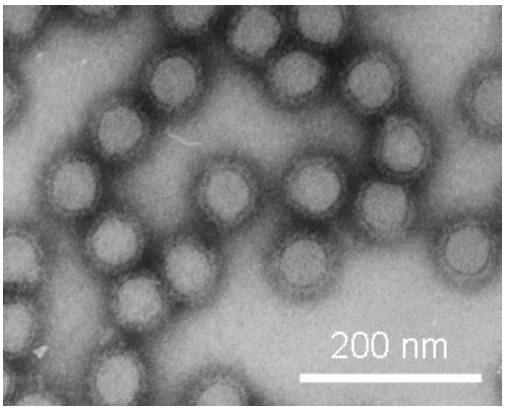
Family	Genus	Subgroup	Virus	Vector	Disease	Geographic distribution	Reference
Bunyaviridae	*Orthobunyavirus*	Bunyamwera	Batai (Calovo)	M	fever	Eurasia, Africa	Bárdos *et al*. 1966
			Bwamba	M	fever	Africa	Smithburn *et al.* 1941
			Cache Valley	M	encephalitic disease	North America	Holden and Hess 1959
			Ngari	M	hemorrhagic fever	Africa	Zeller *et al*. 1996
			Garissa (provisional name)	M	hemorrhagic fever	Africa	Gerrard *et al.* 2004
		California	California encephalitis	M	encephalitic disease	North America	Hammon and Reeves 1952
			Inkoo	M	fever, encephalitic disease?	Eurasia	Oker-Blom and Brummer-Korvenkontio 1985, Putkuri *et al*. 2004
			Jamestown Canyon	M	encephalitic disease	North America	Grimstad *et al*. 1982
			La Crosse	M	encephalitic disease	North America	Thompson *et al*. 1965
			Snowshoe Hare	M	encephalitic disease	North America	Fauvel *et al*. 1980
			Tahyna	M	fever	Eurasia, Africa	Bárdos and Sluka 1963
		Simbu	Oropouche	M	fever, arthralgia	South America	Anderson *et al*. 1961
	Phlebovirus		Rift Valley Fever	S	fever	Africa, Middle East	Daubney *et al*. 1931
			Sandfly fever Naples (Toscana)	S	fever, encephalitic disease	Mediterranean Europe	Ehrnst *et al.* 1985
			Sandfly fever Sicilian	S	fever, encephalitic disease	Mediterranean Europe	Hertig *et al*. 1964
	Nairovirus		Crimean-Congo hemorrhagic	T	hemorrhagic fever	Africa, Russia, Middle East	Gear *et al.* 1982
	not assigned		Bhanja	T	fever, myalgia	Eurasia, Africa	Calisher and Goodpasture 1975
Flaviviridae	*Flavivirus*	Dengue	Dengue	M	hemorrhagic fever	South/Central America, Africa, India, Southeast Asia, Australia	Sabin 1952
		Japanese encephalitis	Japanese encephalitis	M	encephalitic disease	India, East Asia, Southeast Asia	Hayashi 1934
			Kokobera	M	arthralgia	Australia	Doherty *et al.* 1964
			Kunjin	M	encephalitic disease	Australia	Phillips *et al.* 1992
			Murray Valley encephalitis	M	encephalitic disease	Australia	Anderson *et al.* 1951

			St. Louis Encephalitis	M	encephalitic disease	North and South America	Muckenfuss *et al*. 1933
			West Nile	M	encephalitic disease	Eurasia, Africa, India, Australia, North and South America	Smithburn et al. 1940
		Ntaya	Ilheus (Rocio)	M	encephalitic disease	South and Central America	Lopes et al. 1978
		Spondweni	Zika	M	fever	Southeast Asia	Moore *et al*. 1975 and Olson *et al.* 1981
		Yellow fever	Yellow fever	M	hemorrhagic fever	Africa, South America	Stokes *et al.* 1928
			Wesselsbron	M	flu-like disease	Africa	Smithburn *et al.* 1957
		Mammalian tick-borne	Kyasanur Forest Disease	T	encephalitic disease	India	Lyer *et al.* 1959
			Louping ill virus	T	encephalitic disease	UK	Rivers *et al.* 1934
			Omsk hemorrhagic fever	T	hemorrhagic fever	West Siberia	Kharitonova and Leonov 1985
			Powassan	T	encephalitic disease	North America	McLean and Donohue 1959
			Tick-borne encephalitis	T	encephalitic disease	Europe, Russia	Zilber and Soloviev 1946
Reoviridae	*Seadornavirus*		Banna	M	encephalitic disease?	Southeast Asia	Xu *et al*. 1990
	Coltivirus		Colorado Tick Fever	T	fever, myalgia	North America	Becker 1930
			Eyach	T	encephalitic disease?	Europe	Chastel 1998
	Orbivirus		Orungo	M	fever	Africa	Moore *et al*. 1975
Rhabdoviridae	*Vesiculovirus*		Chandipura virus	S	encephalitic disease	India	Bhatt and Rodrigues 1967
Togaviridae	*Alphavirus*	Barmah Forest	Barmah Forest	M	fever, rash, arthritis	Australia	Boughton *et al.* 1988
		Eastern equine encephalitis	Eastern Equine encephalitis	M	encephalitic disease	North, Central, and South America	Feemster 1938, Fothergill *et al*. 1938
		Semliki Forest	Chikungunya	M	fever, rash, arthritis	Southeast Asia, Indian Ocean, Africa	Ross 1956
			Mayaro	M	fever, rash, arthritis	South America	Anderson *et al*. 1957
			O'nyong-nyong	M	fever, rash, arthritis	Africa	Williams *et al.* 1965
			Ross River	M	fever, rash, arthritis	Oceania	Doherty *et al.* 1972
			Semliki Forest	M	fever	Africa	Willems *et al.* 1979, Mathiot *et al.* 1990
		Venezuelan equine encephalitis	Venezuelan equine encephalitis	M	encephalitic disease	South and Central America	Sanmartin-Barberi *et al.* 1954
		Western equine encephalitis	Sindbis	M	fever, rash, arthritis	Eurasia, Australia, Africa	Malherbe *et al*. 1963, McIntosh *et al* 1964
			Western equine encephalitis	M	encephalitic disease	North, Central, and South America	Howitt 1938

network [214]. p62 and E1 are assembled as heterodimers in the endoplasmic reticulum. E3 is cleaved in the Golgi. E1-E2 heterodimers are transported to the plasma membrane, and form trimeric viral spikes. The virus matures by budding at the plasma membrane, from which the virus membrane is derived from [4].

Figure 1.

Electron microscopy image of purified SINV.



Alphaviruses can cause cytopathic effect (CPE) in various mammalian and insect cells, and can be easily propagated into high titers, in temperatures up to 41°C [238], or even as low as 5°C [247]. CPE is characterized by cell rounding, degeneration, lysis, and eventually complete destruction of cell monolayers [238]. Alphavirus infection of vertebrate cells results in inhibition of host protein synthesis, which begins at approximately three hours after infection [228]. Peculiarly, evidence suggests that the transcriptional shutoff caused by the Old World alphaviruses depends on nsP2, whereas C protein determines this phenomenon with the New World alphaviruses [94].

PHYLOGENETIC RELATIONSHIPS AND GEOGRAPHIC DISTRIBUTION

The phylogenetic relationships of alphaviruses, based on the glycoprotein genes (**Figure 3**), resemble those of the suggested serocomplex division [41]. In addition, different alphaviruses are clustered in many respects according to the diseases they cause in humans (**Figure 3**). The origin of alphaviruses is probably in the New World (the Americas) [238]. The general assumption is that Venezuelan equine encephalitis virus (VEEV) diverged from eastern equine encephalitis virus

(EEEV) 1,000 to 2,000 years ago, and SINV and SFV were later introduced to the Old World [250]. A recent report, however, suggests the contrary: alphaviruses may have an Old World origin, instead [137]. Recombination of SINV- and EEEV-like viruses produced western equine encephalitis virus (WEEV) [105].

Currently, WEEV, EEEV, VEEV, and Mayaro virus (MAYV) are exclusively found in the New World, Barmah Forest (BFV) and Ross River virus (RRV) in Oceania, and O'nyong-nyong virus (ONNV) and SFV in Africa. In addition, antibodies to a SFV complex virus have been reported in both birds and humans in Europe, but circulation of such viruses is not established in this continent [152]. Chikungunya virus (CHIKV) infections have been reported in Southeast Asia and Africa, and most recently on the Indian Ocean islands where a massive outbreak began at the end of 2004 [75]. SINV, circulating in Eurasia, Australia, and Africa, is the only human pathogenic alphavirus indigenous in Europe. Interestingly, SINV isolates from Northern Europe and from South Africa are closely related [187], suggesting possible involvement of migratory birds in the distribution of the virus.

Figure 2.

Genomic organization of alphavirus.

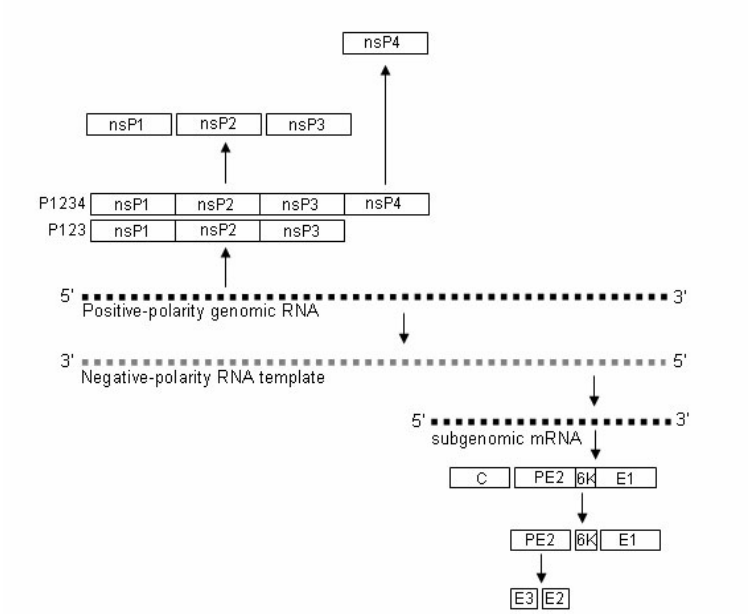
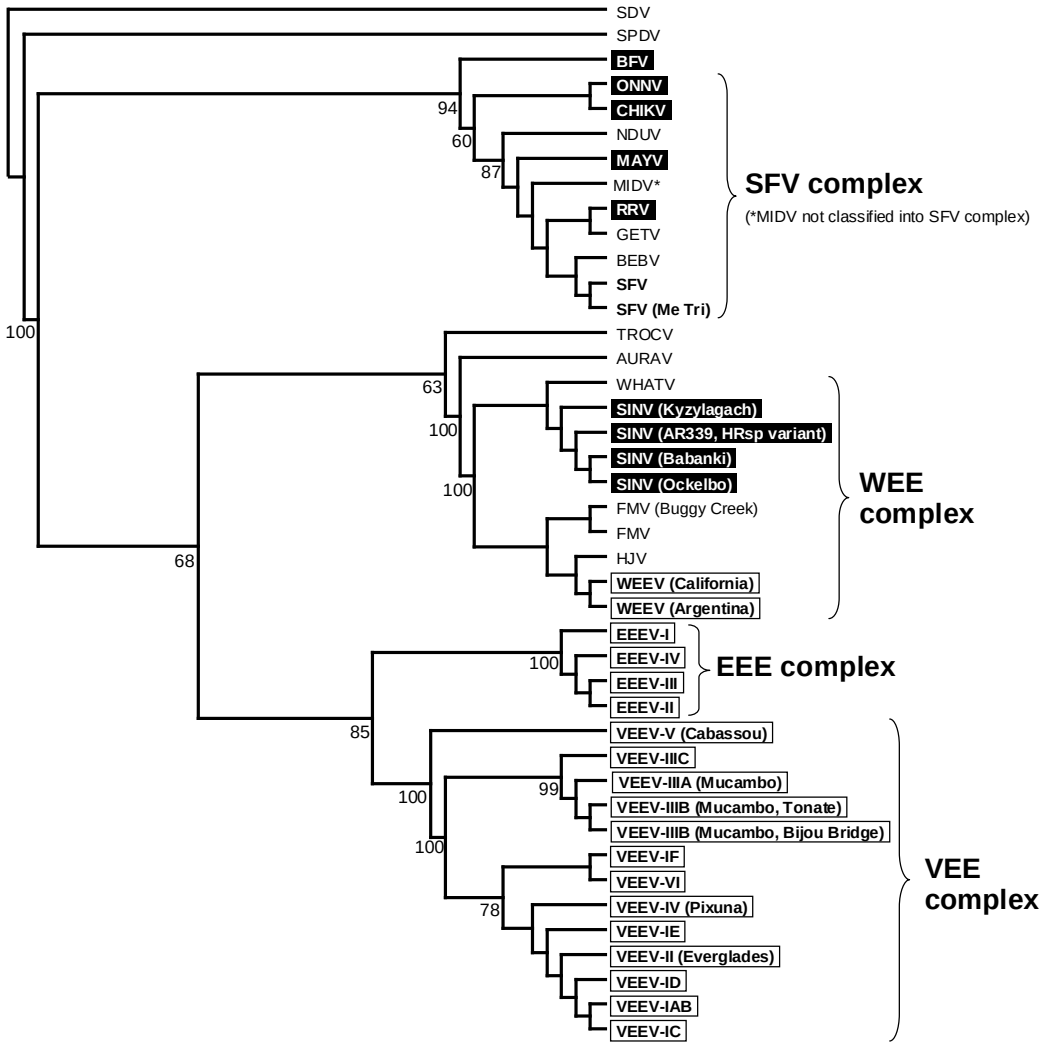


Figure 3.

Phylogenetic relationships of alphaviruses within the E1 region. The viruses and subtypes known to cause disease in humans are highlighted. Black boxes, arthritogenic disease; white boxes, neuropathogenic disease. Phylogenetic tree is based on the nucleotide sequences from E1 region, nucleotides 10,338-11,337; the genome position is given according to the published sequence of the strain AR339 (HRsp variant). The tree was constructed by using neighbor-joining algorithms (NEIGHBOR); 1,000 bootstrap replicates were calculated. Phylogenetic analysis by courtesy of Dr. Tarja Sironen. The following sequences available in GenBank were included in the comparison:

SDV, sleeping disease virus, subtype unknown, France (AJ238578)
SPDV, salmon pancreas disease virus, F93125, United Kingdom (AJ012631)
BFV, Barmah Forest virus, BH2193, Australia (U73745)
ONNV, O'nyong-nyong virus, Gulu, Uganda (M20303)
CHIKV, Chikungunya virus, Ross, Tanganyika (East Africa) (AF192905)
NDUV, Ndumu virus, SAAr221D, South Africa (AF398375)
MAYV, Mayaro virus, TRVL4675, Trinidad (AF398378)
MIDV, Middelburg virus, SA AR749, South Africa (AF398374)
RRV, Ross River virus, T48, Australia (M20162)
BEBV, Bebaru virus, MM2354, Malaysia (AF398376)
GETV, Getah virus, MM2021, Malaysia (AF398377)
SFV, Semliki Forest virus, subtype and origin unknown (X04129)
SFV (Me Tri), Semliki Forest virus, Me Tri, Vietnam (AF398380)
TROCV, Trocara virus, BeAr422431, Brazil (AF252264)
AURAV, Aura virus, BeAr10315, Brazil (AF126284)
WHATV, Whataroa virus, M78, New Zealand (AF398394)
SINV (Kyzylagach), Kyzylagach strain, Leiv65A, Azerbaijan (AF398392)
SINV (AR339, HRsp variant), Sindbis virus, AR339, Egypt (J02363, J02364, J02365, J02366, J02367)
SINV (Babanki), Babanki strain, DAK ArY251, Cameroon (AF398391)
SINV (Ockelbo), Ockelbo strain, Edsbyn 82-5, Sweden (M69205)
FMV (Buggy Creek), 81V1822, Oklahoma (AF398390)
FMV, Fort Morgan virus, 73V1570, Colorado (AF398389)
HJV, Highlands J virus, 64A-1519, Florida (U52586)
WEEV (California), western equine encephalitis virus, BFS1703, California (J03854)
WEEV (Argentina), ag80-646, Argentina (AF398393)
EEEV-I, eastern equine encephalitis virus, lineage 1, 82V2137, Florida (U01034)
EEEV-IV, lineage 4, 430687, Brazil (AF159561)
EEEV-III, lineage 3, 435731, Panama (AF159560)
EEEV-II, lineage 2, BeAn5122, Brazil (AF159559)
VEEV-V (Cabassou), subtype V, CaAr508, French Guiana (AF398387)
VEEV-IIIC, subtype IIIC, 71D1252, Peru (AF398386)
VEEV-IIIA (Mucambo), subtype IIIA, BeAn8, Brazil (AF398383)
VEEV-IIIB (Mucambo, Tonate), subtype IIIB, CaAn410d, French Guiana (AF398384)
VEEV-IIIB (Mucambo, Bijou Bridge), subtype IIIB, Bijou Bridge, Colorado (AF398385)
VEEV-IF, subtype IF, 78V3531, Brazil (AF398382)
VEEV-VI, subtype VI, Ag80-663V, Argentina (AF398388)
VEEV-IV (Pixuna), subtype IV, BeAr35645, Brazil (AF075256)
VEEV-IE, subtype IE, 68U201, Guatemala (U34999)
VEEV-II (Everglades), subtype II, Fe3-7c, Florida (AF075251)
VEEV-ID, subtype ID, 3883, Panama (L00930)
VEEV-IAB, Venezuelan equine encephalitis virus, subtype IAB, Trinidad donkey, Trinidad (L01442)
VEEV-IC, subtype IC, P676, Venezuela (L04653)


SDV
SPDV
BFV
ONNV
CHIKV
NDUV
MAYV
MIDV*
RRV
GETV
BEBV
SFV
SFV (Me Tri)
TROCV
AURAV
WHATV
SINV (Kyzylagach)
SINV (AR339, HRsp variant)
SINV (Babanki)
SINV (Ockelbo)
FMV (Buggy Creek)
FMV
HJV
WEEV (California)
WEEV (Argentina)
EEEV-I
EEEV-IV
EEEV-III
EEEV-II
VEEV-V (Cabassou)
VEEV-IIIC
VEEV-IIIA (Mucambo)
VEEV-IIIB (Mucambo, Tonate)
VEEV-IIIB (Mucambo, Bijou Bridge)
VEEV-IF
VEEV-VI
VEEV-IV (Pixuna)
VEEV-IE
VEEV-II (Everglades)
VEEV-ID
VEEV-IAB
VEEV-IC
94
60
87
100
63
100
100
68
100
85
100
99
100
78
SFV complex
(*MIDV not classified into SFV complex)
WEE complex
EEE complex
VEE complex

RESERVOIRS AND VECTORS

Mosquitoes are the primary vectors for all alphaviruses, and various vertebrate hosts function as their reservoir hosts, depending on the virus and geography.

The hypothesis is that SINV cycles between ornithophilic mosquito species and birds; a cycle resembling e.g. that of West Nile virus. *Culex* [126,167] and *Culiseta* [181] mosquitoes are considered the primary vectors for SINV. Birds might be involved in the natural cycle of SINV as important reservoirs [88,153,169], but spillover to other vertebrates may occur, as well. *Culex annulirostris* and *Ochlerotatus vigilax* are evidently the most important vectors for RRV [87]. The reservoir host for RRV is unknown; however, marsupials and birds may play an important role [111]. *Aedes* species are probably important vectors for BFV [211,248], but its reservoir remains unestablished. ONNV is transmitted by *Anopheles* mosquitoes [254]; the search for its reservoir host has repeatedly failed [189]. The principal vector for CHIKV is *Aedes aegypti*, but during the recent outbreak on the Indian Ocean islands, *Aedes albopictus*, which is usually considered to have a low vectorial competence *in vivo*, was also involved [204]. Monkeys, rodents, birds are the suggested hosts for CHIKV [197]. *Haemagogus janthinomys* mosquitoes are probably the principal vectors, and marmosets (monkeys) the main amplifying hosts for MAYV [116].

WEEV transmission cycle involves *Culex tarsalis* as the primary vector and house finches and house sparrows as the primary amplifying hosts, and to a lesser extent other passerine species, chickens, and possibly pheasants. WEEV also cycles between *Aedes melanimon* and the blacktail jackrabbit [109].

Spiny [42] and cotton [260] rats are the suggested primary hosts for VEEV. It is evident that an epizootic in equines usually precedes human VEEV epidemics. During the epidemics, several mosquito species, including *Psorophora confinnis, P. columbiae, Ochlerotatus sollicitans, O. taeniorhynchus*, and different *Culex* species, can function as vectors for the virus [249], whereas the enzootic vectors of VEEV are all members of the *Culex melanoconion* subgenus [82].

The North American and South American EEEV transmission cycles differ from each other. The majority of mosquito isolations of EEEV in North America have been from *Culiseta melanura* with several studies demonstrating either neutralizing

antibodies or the virus from passerines [217]. Thus, EEEV is considered to cycle between *Culiseta melanura* and passerine birds in North America. The virus has also been isolated from *Aedes albopictus* [172], which is a cause for concern, since the species is widely spread in the US. In South America, the EEEV transmission cycle is not established in detail. However, mosquitoes in the *Culex melanoconion* subgenus are probably involved in the transmission, and rodents and marsupials might function as vertebrate hosts for the virus [217].

ALPHAVIRUSES AS HUMAN PATHOGENS

Human pathogenic alphaviruses cause either an arthropathic disease (BFV, CHIKV, MAYV, ONNV, RRV, SINV) or a neuropathogenic (encephalitic) disease (EEEV, VEEV, WEEV) (**Figure 3**). In addition, SFV can cause a fever syndrome, sometimes including arthralgia, reported in a single natural epidemic [166], and as a fatal laboratory-acquired infection [253]. Neuropathogenic alphaviruses are considered potential agents for bioterrorism [68]. Human pathogenicity of SINV and SFV is noteworthy, since they are widely used as models in virus research, and considered potential tools for gene therapy [156].

RRV is the most commonly reported alphavirus infection on a regular basis, with approximately 1,500-8,500 cases per year in Australia (1993-2006) [3]. The annual number of BFV cases in Australia has been roughly 1,000 [3]. Clinical SINV infection is almost exclusively found in Northern Europe, with a few tens to over 1,300 cases per year in Finland [2]. For unknown reason, SINV outbreaks have peculiarly occurred every seven years in Northern Europe since 1974 [31]. The last outbreak took place in 2002 [2].

Millions of people manifested with ONNV infection in Uganda in the 1950s [255], and the virus re-emerged there in the 1990s [209]. Sporadic CHIKV outbreaks were reported in Africa and Southeast Asia before the massive outbreak in Indian Ocean with several hundred thousand people infected in 2004-2006 [75]. Several small MAYV outbreaks have occurred in South America [43,199,234].

In the US, 0-21 cases of EEEV and 0-172 cases of WEEV were reported per year during 1964-2005 [1]. VEEV has occurred in the Americas as periodic outbreaks, and one of the largest VEEV epidemics involved an estimated 75,000 to 100,000 people in 1995 in Venezuela [251].

In conclusion, many of the human pathogenic alphavirus infections tend to have a fluctuating incidence with occasional, sometimes massive, outbreaks followed by years of silence. RNA viruses have very high mutation rates. Virus-encoded RNA-dependent RNA polymerase, which is responsible for genome replication in positive-stranded RNA viruses, has no proof-reading activity. These aspects enable alphaviruses to transform from endemic virus strains to epidemic strains, and facilitate their adaptation to hosts and vectors.

3. CLINICAL REVIEW OF NEUROPATHOGENIC ALPHAVIRUSES

Neuropathogenic alphavirus infections with an established association with clinical disease are reviewed below. All these viruses are major equine pathogens, but occasionally equine epizootics expand to human epidemics.

EASTERN EQUINE ENCEPHALITIS VIRUS

EEEV infections are reported in the eastern and southern parts of the US [1]. Since the first outbreak in 1831 in Massachusetts [108], infections in horses consistent with the clinical picture of EEEV infection were reported in several instances in North America. The human EEEV outbreak in the summer of 1938 in Massachusetts was the first time any equine virus has verifiably caused a symptomatic infection in humans [79,85,98]. Thus far, the Massachusetts outbreak, and the one in New Jersey in 1959 [99], have been the most prominent EEEV outbreaks in humans with several tens of cases diagnosed in laboratory. Even though the disease is rare, EEEV infection is clinically important due to its high mortality and severe symptoms. The clinical course of symptomatic EEEV infection is the most severe of the three equine encephalitides. Comparison of the pathogenesis of EEEV and WEEV in mice has shown that although both are highly neurotropic, EEEV is far more virulent [150].

With North American EEEV strains it has been demonstrated that 3-6% of infected humans are symptomatic [100]. A symptomatic EEEV infection typically manifests with high fever, headache, nausea and vomiting, malaise, stupor, convulsions, meningeal irritation (neck stiffness) and nonfocal weakness [62,80,98,112]. In addition, confusion, myalgia, arthralgia, signs of dysfunction in upper (positive

Babinski sign) or lower (focal weakness) motor neurons, chills, and seizures are common [62]. Infants sometimes present with a peculiar edema around the eyes and in the upper extremities [80].

In fatal cases, the duration of the acute illness is usually less than one week; in the survivors the duration is usually several weeks [98]. Children are most often affected by clinical EEEV infection [98]. Serious or fatal symptoms caused by EEEV infection are most often seen in small children [80]. The onset of symptoms is typically more sudden in infants than in older patients [80,98].

At the early stage of the disease, cerebrospinal fluid (CSF) shows pleocytosis, and an elevated protein concentration and red-cell count in most cases [62]. In peripheral blood, leukocytosis and hyponatremia are typical [62]. Electroencephalography typically shows generalized slowing and disorganization of the background [62]. In neuroradiologic examinations abnormal findings are often seen, especially in the areas of basal ganglia and thalami [62,157,198]. Evidence suggests that high white-cell count in CSF and severe hyponatremia at the early stages of the disease can be a sign of poor prognosis [62].

Long-term, sometimes even fatal, sequelae are common in patients who survive the acute phase of the EEEV infection [12,98]. There are various long-term effects, such as mental retardation or deficiency, lack of emotional control, aphasia, paresis, cranial nerve palsies, deafness, recurrent convulsions, or epilepsy, which all can continue for years or become permanent [12]. Of the eight patients that were followed for nine years after the outbreak in Massachusetts in 1938, two died and only one had a complete recovery [12], reflecting the poor long-term prognosis of the infection. Nine years after this outbreak the overall mortality rate was 90% in the 34 patients reported [12]. The mortality among the 36 patients who had acquired EEEV infection during 1988-1994 in the US was 36%; in addition, 35% manifested with moderate or severe disability [62].

WESTERN EQUINE ENCEPHALITIS VIRUS

WEEV was first isolated from *Culex tarsalis* mosquitoes in Yakima Valley in the North-Western US in 1941 [107]. Human WEEV infections are exclusively reported in the Western Hemisphere, and most infections occur in the western and central US states [1].

Generally, acute WEEV infection is characterized by fever, headache, drowsiness, signs of meningeal irritation (neck stiffness), nausea, vomiting, muscle aches, and more rarely, paresis and convulsions [139]. In infants, in contrast, convulsions are common, and the progression of the disease more rapid [48,74]. During the acute phase, white blood cell count is normal or slightly elevated in peripheral blood; CSF glucose level is normal, whereas protein level and white blood cell count can be elevated [139]. Lateralized abnormalities in the electroencephalogram are possible, but not typical [230].

After the encephalitic symptoms have subsided, some patients manifest with Parkinson-like symptoms [177,216]. Other long-term sequelae vary from difficulty in coordination and speech to muscle rigidity and cognitive impairment [177]. Psychiatric sequelae are possible [93]. Even intracranial calcifications have been described in a pediatric case five months after the infection [225].

The severity of the sequelae is possibly related to the duration of unconsciousness at the acute phase [177]. The detected antibody response and the severity of the acute illness do not seem to correlate [48]. The mortality rate of WEEV infection is significantly lower than in EEEV infection, approximately 3-5% [35,74]. The ratio of subclinical to clinical infections ranges from 50:1 in children to 1000:1 in adults [74]. Transplacental transmission of WEEV can occur [52,220].

VENEZUELAN EQUINE ENCEPHALITIS VIRUS

VEEV is present in Central America, and northern and eastern parts of South America [249]. Epidemics and equine enzootics occur periodically, sometimes in a massive scale [251]. VEEV subtypes IA-C that have the potential to cause an epizootic, cause more severe, and highly fatal infection in equines than the enzootic subtypes (ID-F, II-VI). However, the severity of the human disease does not necessarily depend on the subtype, and is often self-limited. A recent study provides evidence that only a few site mutations in the E2 region can cause transformation of the VEEV from an enzootic to an epidemic strain [9]. In addition to its innate neurovirulence, the potential of a VEEV strain to cause encephalitis seems to be related to its ability to replicate extra-cerebrally [249].

The onset of the acute disease is usually sudden, but the clinical course may vary considerably. Most of the patients manifest with an influenza-like infection, with a

few days of fever, pharyngitis, and general symptoms, whereas some infections are much more severe, either characterized by convulsions, shock, and coma or an encephalitic form of illness with central nervous system manifestations [72]. In general, most patients feature fever, headache, muscle pain, and nausea or vomiting [60,64,132]. Signs of meningeal irritation (neck stiffness) or focal neurologic manifestations are not seen in most patients [64]; neurologic disease appears in <15% of the serodiagnosed cases [249]. Similar to EEEV and WEEV, children manifest with a more severe form of illness than adults, and their fatality rate is higher [72]. Lymphopenia in the peripheral blood is typical for the acute phase [64].

In the less severe cases, defervescence takes place rapidly, within a few days [64], and other symptoms usually subside within a week [249]. However, recurrence of symptoms, particularly fever and headache, can occur [72,132]. Sequelae of a severe VEEV infection can include various neurologic deficits [140]. VEEV can vertically infect a fetus, resulting in stillbirth, spontaneous abortion or a severe neurologic infection of the infant [252].

PATHOGENESIS OF NEUROPATHOGENIC ALPHAVIRUS INFECTIONS

Various histopathologic changes take place in brain tissue during EEEV infection. Polymorphonuclear and mononuclear cells accumulate in perivascular and meningeal regions and neuron destruction occurs [80,112,123,256]. Small thrombi are seen in vessels [80]. In addition to brain tissue, EEEV infects various organs in a hamster model, including heart, lung, liver, kidney, and spleen [193]. In a hamster model, brains show considerable hyperemia, vasculitis, and subependymal and subarachnoidal hemorrhages [193]. Small thrombi have also been reported to form in internal organs [80]. Studies with mice have demonstrated that EEEV replication takes place in metaphyseal osteoblasts at the early stage of the infection, possibly explaining the vulnerability of the young to the more severe infection [243]. Perivascular infiltration and multifocal necrosis, especially in the deep grey matter of the brain [5], as well as edema and meningeal lesions [35] are seen in fatal WEEV cases.

Studying fatal cases of VEEV infection has revealed that histopathologic lesions, especially edema, congestions, and meningitis, are seen in the central nervous

system without exception [59]. Histopathologic lesions in other organs typically involve follicular necrosis in the spleen and lymph nodes, congestion/edema and interstitial pneumonia in the lungs, hepatocellular degeneration in the liver, and lymphoid follicle necrosis, congestion, and edema in the gastrointestinal tract [59]. Destruction of lymphocytes, especially in the young, is considered to play an important role in the pathogenesis of VEEV infection [72]. A mouse model has shown that after the infection, and subsequent clearance of VEEV from the visceral organs, the virus begins to appear in the brain, first in the olfactory tracts [58]. The E2 glycoprotein appears to play an important role in the pathogenesis of VEEV at a very early step, by rendering its spread to the draining lymph node and beyond [10,58,162]. After subcutaneous inoculation of mice, VEEV replication takes place in the lymph node draining [10,162]. It is notable that SINV and SFV, which cause an arthropathic disease in humans, are neuropathogenic in mice in an age-dependent manner [78,121].

4. CLINICAL REVIEW OF ARTHRITOGENIC ALPHAVIRUSES

Arthritogenic alphavirus infections with an established association with clinical disease are reviewed below. Except for BFV and SINV, all arthritogenic alphaviruses are members of the SFV serocomplex. SFV infection is not reviewed here due to its rarity and poorly described clinical manifestations [166,253].

SINDBIS VIRUS

SINV was first isolated in 1952 from a pool of *Culex pipiens* and *C. univittatus* mosquitoes collected from the village of Sindbis in the Nile river delta in Egypt [232]. The first human isolations took place in Uganda in 1961, but possible clinical manifestations of the individuals were not reported [257]. The virus was recovered for the first time from an acutely ill patient in South Africa (Girdwood strain) in 1963 from the vesicle fluid of skin lesions [164]. The virus was associated as the causative agent of a rash-arthritis syndrome [168].

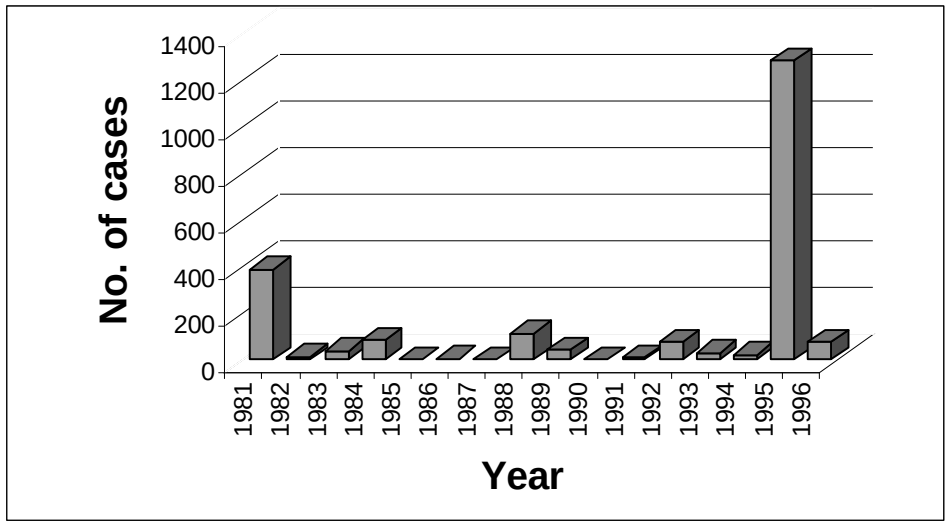
In Europe, antibodies to SINV were found for the first time by screening human sera in 1965 in Finland [29], but a connection to a clinical disease remained to be shown. In the late summer of 1974, in the Eastern Finland village, Ilomantsi, a

general physician noticed a peculiar rash and simultaneous joint pain in several patients within a few months time. The syndrome was named Pogosta disease, according to the name of the village. Pogosta disease was serologically associated with SINV infection in 1980 [28].

SINV or antibodies to SINV are widely found in insects and vertebrates in Eurasia, Africa, and Oceania. However, clinical disease caused by SINV infection is almost exclusively reported in Northern Europe, with only sporadic cases elsewhere. In Northern Europe, the virus has a peculiar epidemiologic cycle: since 1974 an epidemic has occurred every seven years, latest in 2002 [31] (**Figure 4**). The largest outbreak thus far in Finland took place in 1995, with over 1,300 serodiagnosed cases [2]. Similar disease is also found in Sweden (Ockelbo disease) and Russian Karelia (Karelian fever); SINV has been isolated from mosquitoes in both [160,181]. In Sweden, the number of cases has been considerably lower. For example, during the 2002 epidemic in Finland, with approximately 600 serodiagnoses, only four patients were serodiagnosed in Sweden (Sirkka Vene, the Swedish Institute for Infectious Disease Control, personal communication). From Russia, the number of SINV infections is not available, but it is plausible that the endemic zone in Eastern Finland must continue over to the Russian side of the border.

Figure 4.

Annual number of serodiagnosed Pogosta disease cases during 1981-1996. Data from Brummer-Korvenkontio *et al*. 2002 [31].



Brummer-Korvenkontio *et al.* have previously screened SINV antibodies in Finland [31]. The incidence of SINV in 1980-1996 was 2.7/100,000 in Finland, with the highest incidence in the provinces of North Karelia and Central Finland. The seroprevalence in over 600 individuals without joint manifestations in Ilomantsi, North Karelia, Eastern Finland in 1982 was 39%, being considerably higher than in 1980, due to the disease epidemic in 1981. The seroprevalence in 5000 pregnant women in their first trimester in February 1992 throughout the country was 0.6%. 17% of game mammals (N=41) and 30% of birds (N=80) were positive in SINV antibody testing in Ilomantsi during 1981-1983.

Maculopapular (itchy) skin rash, fever, and joint symptoms, particularly in wrists, hips, knees, and ankles, are the hallmarks of the acute clinical picture of SINV infection, sometimes accompanied by nausea, general malaise, headache, and muscle pain [76,239,242]. Infectious parameters are typically normal [239]. There is one case report of cutaneous hemorrhagic vesicles during the acute phase of SINV infection [103].

Evidence suggests that SINV infection can cause prolonged joint manifestations. In a questionnaire study and telephone interview carried out with Ockelbo disease patients in 1985 in Sweden, 24% (21/86) of the patients reported arthralgia three to four years after infection, and the symptoms had a temporal linkage with the infection. Of the 21 patients 14 complained arthralgia 5-6 years after infection, and the symptoms mostly affected hands and feet; arthralgia was present often in the same joints that manifested with most severe symptoms at the acute phase [180,182]. In a smaller study in 1989, 31% (5/16) of the patients with Ockelbo disease complained joint ache when interviewed by telephone 2.5 years after infection [242]. Chronic arthritis and Pogosta disease were linked in a case report in 2000 [158], and a clinical examination of 26 patients 2.5 years after serologically confirmed SINV infection showed that 8% (2/26) of the patients had arthritis, and 42% (11/26) had either osteoarthritis, fibromyalgia or occasional arthralgia [133].

ROSS RIVER VIRUS

RRV is present exclusively in Oceania, where the first human outbreak took place in the 1920s, in South-Eastern Australia [184]. The association between RRV and the disease was shown much later with serology [67], and eventually by virus

isolation [65]. The virus is endemic in the coastal regions of Australia, particularly in the north-eastern parts of the country [163]. The infection is also known as "epidemic polyarthritis".

RRV disease is found in all age groups, but most commonly in the age group 30-49 years [50]. Patients with acute RRV infection present with joint symptoms, fatigue, lethargy, muscle pain, skin rash, fever, and headache [50,83,218]. Knees, wrists, ankles, and fingers are most commonly affected [50,110,178]. A single case of acute glomerulonephritis during the acute phase of RRV infection has been described [91], as well as another case of reactive arthritis and vasculitis as a consequence of the infection [81].

Prolonged joint sequelae are seen after RRV infection. In a self-administered questionnaire study, 57% of the of 255 RRV disease patients infected in 1988-89 reported joint pain at 24-42 months post infection [50], whereas in another study in 1992-93, 12% (51/436) of the patients complained joint pain and 3% (14/436) swollen joints 15 months after infection [218]. In a prospective clinical follow-up in 1998, 68% (32/47) of the patients complained joint pain 2.3–6.5 months after infection [110]. In a quality-of-life questionnaire study with 67 RRV disease patients in 1997-2000, patients with persistent symptoms usually had other rheumatic conditions as well, suggesting that previous reports might have overestimated the frequency of persistent joint symptoms [178].

BARMAH FOREST VIRUS

BFV was first isolated in 1974 from *Culex annulirostris* at Barmah Forest in South-Eastern Australia [165]. BFV was later isolated from acutely ill patients [196]. The virus is endemic in northern and north-eastern Australia, and parts of western Australia [83].

Maculopapular rash, joint pain, myalgia, fatigue, lethargy, fever characterize acute BFV infection, often accompanied by headache [146,163,196]. The most commonly affected joints are knee, ankle, wrist, fingers, hip, shoulder, and neck [17]. Similar to RRV infection, a single case of glomerulonephritis after BFV infection has been reported [127].

In a self-administered retrospective questionnaire survey of 53 patients in 1995 in Australia, arthralgia or other symptoms of BFV disease were found to last longer than six months in half of the patients [17], but more recent data evidently suggest that in fact only 10% of BFV disease patients feature persisting arthralgia [83].

CHIKUNGUNYA VIRUS

CHIKV has recently drawn attention due to the massive outbreak that began at the end of 2004, involving several million people on the Indian Ocean islands, and parts of India [75]. For instance, an estimated 1/4-1/3 of the residents of Réunion Island, to where the virus was now introduced for the first time, acquired the infection during the outbreak. The outbreak also resulted in an epidemic of a few hundred patients in Northern Italy in 2007, to where the virus was probably introduced by a visitor from India, and then transmitted to inhabitants by *Aedes Albopictus* mosquitoes. Prior to the Indian Ocean outbreak, several smaller CHIKV epidemics have occurred in Africa and especially in Southeast Asia. The primary differential diagnostic alternative of CHIKV infection is dengue virus infection; they partially share the same geographical distribution, and their clinical pictures resemble each other in many respects [183].

The clinical picture of the acute phase of CHIKV infection consists of fever, agonizing arthralgia, myalgia, macular or maculopapular skin rash, and sometimes nausea, conjunctivitis, and headache [61,183,231]. Of the joints, wrists, ankles, and phalangeal joints are often affected [231]. In some patients, leukopenia, thrombocytopenia, or elevated aminotransferase levels can be observed at the acute phase [231]. Various ocular manifestations can occur [135]. Acute myocarditis has been described in a case report [171]. In general, the symptoms of CHIKV infection are the same as in SINV infection, but in a much more severe scale; even fatal cases have been reported [44].

In the examination of 20 South-African patients with Chikungunya fever 4-16 months after infection in 1976, 60% had at least moderate pain in joints, and 70% (14/20) at least moderate morning stiffness [128], whereas in another study in 1977, 18% (5/28) patients manifested with arthritis 20 months after CHIKV infection [86]. A single case report has shown a possible linkage between CHIKV and destructive arthropathy of metatarsophalangeal joints a few years after infection [24].

Serological evidence suggests that CHIKV infection might be a risk factor for Burkitt's lymphoma [240].

O'NYONG-NYONG VIRUS

The clinical disease caused by ONNV was first described in Uganda during a massive outbreak with an estimated two million patients in 1959-1961. During the outbreak, the virus was isolated from both acutely ill patients and *Anopheles* mosquitoes [254,255]. Peculiarly, the virus disappeared for 35 years to re-emerge in 1996, again in Uganda [210]. The ONNV strains isolated during these two epidemics were closely related [136]. In addition, an ONNV outbreak occurred in 2003 in a refugee camp in Ivory Coast [199], and a single case was reported from Chad in 2004 [19].

Acute ONNV infection has a sudden onset with slightly elevated temperature, joint pain, muscle pain, headache, itchy skin rash, and sometimes lymphadenopathy and retro-orbital pain [131,221]. Knees, elbows, wrists, fingers, and ankles are typically affected [131,221]. Severity of joint manifestations varies from vague to excruciating [221].

Possible persistence of joint manifestations in ONNV infection is poorly known. Shore reported that joint symptoms "caused the disease to be protracted in some patients" during the first ONNV outbreak [221]. In a retrospective study three months after the infection, the median duration of joint pain was 7 days (range, 1–90 days) [131].

MAYARO VIRUS

MAYV, present in South America, is the only arthritogenic alphavirus in the New World. The virus was first isolated in Trinidad in 1954 from febrile patients [6]. In the 1950s, MAYV outbreaks were described also in the state of Pará in Brazil [43] and in Bolivia [215]. Thereafter, small sporadic epidemics have been reported every now and then. Although widespread in South America, human MAYV infection usually occurs only in rural conditions.

Patients with acute MAYV infection present with fever, (sometimes severe) joint pain, headache, chills, dizziness, myalgia, maculopapular skin rash, eye pain, and

lymphadenopathy [6,43,199,234]. The onset of the disease is usually abrupt, and wrists, fingers, ankles, and toes are commonly affected [199]. Leukopenia is typical at the acute phase [199].

A few case reports have linked Mayaro fever with recurrent [233] and persisting [234] arthralgia.

PATHOGENESIS OF ALPHAVIRUS ARTHRITIS

Pathogenesis of joint manifestations in the arthritogenic alphavirus infections is inadequately understood, but several mechanisms have been suggested. SINV replication has been detected within connective tissue adjacent to articular joints in a mouse model [114], and RRV can be propagated in human synovial cells in vitro [124]. Circulating immune complexes have been detected in the sera of SINV-infected patients [125]; however, studies with RRV have shown contrary evidence [90].

Linn and coworkers have shown that RRV persists in synovial macrophages [147], and later demonstrated this also with SINV and other alphaviruses [229]. Their further studies support the idea that persisting joint symptoms in certain individuals could be due to inability to produce adequate anti-alphavirus cytotoxic T lymphocyte activity [149]. On the other hand, viral tropism might play an important role, since mouse models have shown that the primary targets of RRV are musculoskeletal tissues [175]. Interestingly, evidence suggests that RRV uses the collagen IV binding β1 integrin α1β1, as a receptor in mammalian cells; the collagen receptors and their genetic variation might be related to the pathogenesis [148]. As for genetic determinants, evidence suggests that human leukocyte antigen haplotype DR7 is more prevalent among RRV patients [92]. A recent study indicates that complement activation occurs in response to RRV infection and enhances the severity of the disease in mice [174].

In a recent study on patients with CHIKV infection, CHIKV antigen could be detected by immunohistochemistry in muscle satellite cells, which are myogenic precursor cells considered the main cell type responsible for postnatal muscle growth and repair [192]. Notably, CHIKV antigen could not be detected in muscle fibers, infiltrating cells, or endothelial cells [192].

5. ALPHAVIRUS DIAGNOSTICS, TREATMENT, AND PREVENTION

The laboratory diagnosis of all alphavirus infections is primarily based on serology, i.e. detection of specific IgM antibodies and/or seroconversion of IgG between paired samples. The possible serological methods include EIAs, hemagglutination inhibition (HI) test, neutralization tests (NT), and immunoblot [22,36,47,120,179,207,242]. Various PCR techniques [118] and virus isolation can also be used. However, the viremic window in alphavirus disease is usually brief, which can limit their routine use. A special reverse transcriptase polymerase chain reaction (RT-PCR)-EIA technique, which is able to distinguish between EEEV, WEEV, and VEEV, has also been described [246].

No specific antiviral treatment is available for any of the alphavirus infections. Thus, patients are only treated symptomatically. The treatment of neuropathogenic infections focuses on supporting vital functions, and on anticonvulsion. Symptoms of arthritogenic infections can be relieved with non-steroidal anti-inflammatory agents, and rash with antihistamins. *In vitro* studies provide potential for the drug development in the future. A recent study on mice provided evidence that certain form of steroids (*seco*-pregnane steroids) have a potential antiviral effect against alphaviruses [141]. Short interfering RNAs may have antiviral effect against VEEV [188], and the rat zinc-finger antiviral protein expression has been reported to inhibit SINV replication after virus penetration and entry [21]. Ribavirin and human recombinant IFN-alpha have shown an anti-CHIKV and anti-SFV effect *in vitro* [25]. Ribozymes are suggested to inhibit alphavirus replication [219,262].

Avoiding mosquito bites is the cheapest, but often unreliable way to protect oneself from alphavirus infection. Human vaccine prevention is currently available for VEEV and EEEV. Against VEEV, an attenuated vaccine TC-83 and a formalin-inactivated vaccine C-84 have been used [33,70]. Currently, a promising, live-attenuated VEEV vaccine V3526 is being tested [202]. The current EEEV vaccines are inactivated products [49] that have low immunogenicity, requiring multiple inoculations and periodic boosters. No human WEEV vaccine is available; however, adenovirus-vectored WEEV vaccine has shown promise in mouse experiments [14]. A formaldehyde-inactivated RRV vaccine has been preclinically promising [130]. Live CHIKV vaccine has undergone a phase II study

in which it was proven reasonably safe and immunogenic [71]. There are no clinically used or pre-clinically promising/safe vaccines for the other human pathogenic alphaviruses. Production of alphavirus infectious clones could be used to create live attenuated vaccine candidates in the future [200].

6. OTHER ARTHRITOGENIC VIRUS INFECTIONS

In addition to alphaviruses, several other viruses are known or proposed to cause joint manifestations. One of the major viral arthritides is human parvovirus B19 (genus *Erythrovirus*, family *Parvoviridae*) infection. B19 infection causes a variety of clinical manifestations. In children, a peculiar erythema infectiosum is commonly present during the acute phase, but arthropathy is rarely seen [186]. In adults, however, a symptomatic infection is characterized by polyarthritis that may persist for months, and is more common in women than men [258]. Metacarpophalangeal joints, knees, and wrists are commonly affected [203]. Hematological or internal organ complications, or intrathecal infection may occur [26].

Hepatitis C virus (genus *Hepacivirus*, family *Flaviviridae*) can often manifest with extra-hepatic syndromes, including arthralgia (9% of the patients) or arthritis (4%) [34]. Joint manifestations are sometimes seen in hepatitis B virus (genus *Orthohepadnavirus*, family *Hepadnaviridae*) infection, as well, and its pathogenic role in rheumatoid arthritis has been investigated [55].

Due to vaccine coverage, rubella virus (genus *Rubivirus*, family *Togaviridae*) infection is rarely seen anymore in the western world. Arthritogenic manifestations are typical of rubella infection, particularly in women. Arthritis has been suggested as a complication of rubella vaccination [237]. Joint manifestations are accompanied by rash and lymphadenopathy. Congenital rubella infection leads to severe birth defects [13]. Mumps virus (genus *Rubulavirus*, family *Paramyxoviridae*) is predominantly found in countries with poor vaccine coverage, as well. Patients with mumps typically present with bilateral parotid swelling, fever, and headache [104]. Arthropathy is more a complication than a typical manifestation in mumps virus infection [101]. Other potential complications are e.g. orchitis and meningitis [104].

Numerous clinical manifestations and associated infections are seen in patients with human immunodeficiency virus (genus *Lentivirus*, family *Retroviridae*) infection. Compared to general population, various rheumatic manifestations are overrepresented in these patients [56]. Human T-lymphotropic virus type 1 (genus *Deltaretrovirus*, family *Retroviridae*) infection can present with a chronic arthropathy, which can be severe and destructive [185]. Joint symptoms are sometimes seen in hantavirus infections, as well [138]. Certain herpesviruses have been suggested as causative agents of joint manifestations, including Epstein-Barr virus [53], cytomegalovirus [51], and varicella zoster virus [225].

AIMS OF THE STUDY

Serological evidence for the association of SINV and clinical syndrome manifesting with rash-arthritis was first noted in Northern Europe, in the early 1980s. Although SINV is prevalent in insects and vertebrates all around Eurasia, Africa, and Australia, clinical disease in humans, known as Pogosta disease in Finland, has almost exclusively been reported in Northern Europe. The molecular biology of SINV is well known. In contrast, the clinical virology and epidemiology are less investigated.

The specific aims of the study were to:

- Develop and evaluate methods for the serodiagnostics of Pogosta disease
- Establish the etiologic agent of Pogosta disease
- Characterize the clinical picture of acute Pogosta disease
- Study the long-term prognosis of Pogosta disease
- Study the clinical virologic aspects involved in SINV infection
- Characterize epidemiology of SINV infection in Finnish population
- Search for factors contributing to the seven-year epidemiologic pattern of Pogosta disease in Finland

MATERIALS AND METHODS

PATIENTS AND MATERIALS

PATIENTS AND PATIENT SAMPLES (**II**, **III**, **IV**)

Original publications **II**, **III**, and **IV** describe studies on a cohort of patients (**Figure 5**) recruited to the study in eleven health care centers in North Karelia and in Kuopio University Hospital during a Pogosta disease outbreak in July-October of 2002. Altogether 131 patients with clinically suspected acute Pogosta disease participated in the study in eleven health stations in the province of North Karelia and in Kuopio University Hospital, Finland. Of these patients, 86 had an acute, serologically confirmed SINV infection. The acute-phase samples included serum samples, whole blood samples in tubes containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant, and skin lesion biopsies.

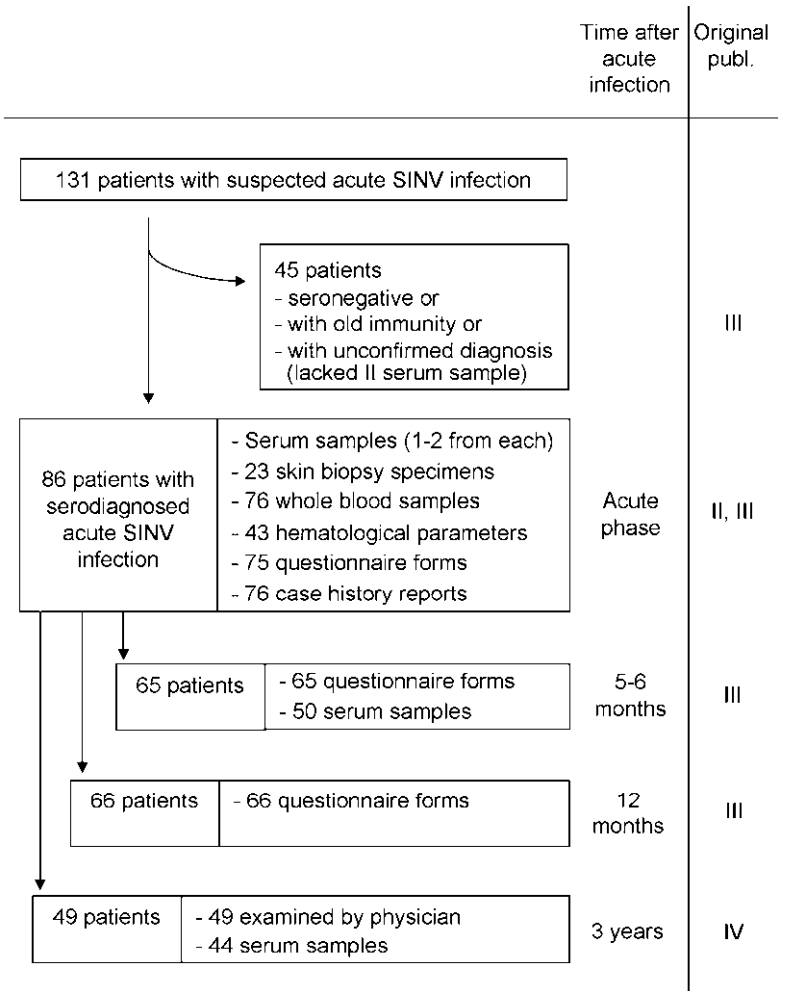
Case history reports on physician's examination and basic blood parameters were also available in most cases at the acute phase. New serum samples were collected 1-2 weeks and 5-6 months post infection from most patients. A questionnaire study was carried out to collect descriptive information on the clinical picture of Pogosta disease. The patients filled a questionnaire at the acute phase, and 5-6 months and 12-13 months post infection. The questionnaires focused on the prevalence, onset, duration and anatomical location of different symptoms, and the patients' subjective experience.

At three years post infection (median 39.3 months) in November-December 2005, 81 of the patients could be re-invited to the study for physical examination and interview (**IV**). Serum samples were retrieved when possible. At this stage, 49/81 (60%) of the patients participated. In joint examination (performed by a senior rheumatologist), 72 joints were assessed for tenderness and 70 for swelling, according to the EULAR handbook of clinical assessments [241]. Patients were also

screened for spondyloarthropathy with the European spondyloarthropathy study group criteria [69]. The general examination (performed by a general physician) included cardiac and pulmonary auscultation, abdominal palpation, examination of skin and mouth, and palpation of lymph node areas. Standardized interview was carried out by physicians. The interview dealt with the patient's medical history, current subjective joint symptoms (arthralgia or tenderness in joint movement), and possible temporal association of current symptoms with acute SINV infection.

Figure 5.

Patients and material in original publications **II**, **III**, **IV**. The original cohort of 131 patients was recruited during the 2002 Pogosta disease outbreak.



SERUM SPECIMEN LIBRARY (**I**, **V**)

Specimen library consisting of serum samples sent to the Department of Virology of the Helsinki University Central Hospital Laboratory during 1997-2003 for SINV antibody testing was used for the development and evaluation of SINV enzyme immunoassays (EIA) (**I**), and for seroprevalence analysis (**V**).

For the EIA development (**I**), paired serum samples taken at approximately two-week intervals from Finnish Pogosta disease suspected patients during 1997–1998 were selected. These included paired samples from 46 patients with acute SINV infection confirmed with at least a four-fold rise in SINV HI antibody titers, and 40 first samples from individuals in whom the HI test remained negative in paired samples. A third independent panel was randomly selected to determine specificity of the assays, consisting of 42 first samples from patients in whom the SINV HI test remained negative in two samples taken in 10–103 days interval.

All samples from 10/6/1999 to 5/8/2003 were included in the analysis of the seroprevalence of SINV in Finnish population (**V**). If a patient had multiple samples, only the most recent sample was included in the study, and those with acute SINV infection (IgM-positive samples) were excluded.

DATABASE MATERIAL (**V**)

Since 1995, Finnish clinical microbiology laboratories have notified laboratory-confirmed diagnoses of SINV infection (by antibody detection) to the National Infectious Disease Registry maintained by the National Public Health Institute (KTL). Each notification includes information on the date of specimen collection, date of birth, sex and place of treatment. Using this information multiple notifications for the same person which are received within a 12-month period are combined as a single case. Data reported from January 1995 to December 2003 were included in the analysis of incidence of SINV in the Finnish population.

RESIDENT GROUSE BLOOD SAMPLES (**V**)

Blood samples from grouse were collected by hunters during hunting seasons between 10 September and 31 October 2003, and 10 September and 31 October 2004. Grouse belong to the order *Galliformes*, and family *Tetraonidae*; the species

from which blood samples were collected were Capercaillie (*Tetrao urogallus*), Black Grouse (*Tetrao tetrix*), Hazel Grouse (*Bonasa bonasia*), and Willow Grouse (*Lagopus lagopus*). The blood was absorbed into small filter paper slips, dried, and stored individually in small plastic bags at -20°C. The hunters determined each bird's sex and whether it was a juvenile (born the same year) or an adult.

MIGRATORY BIRD BLOOD SAMPLES (**V**)

In 2004, blood samples were collected from migratory birds in two bird observatories during their spring migration: in Jurmo Island (N 59°50', E 21°36'; EUREF-FIN) between 18 and 19 May and in Tauvo (N 64°49', E 24°37') between 24 and 27 May. In 2005, blood samples were also collected in two different bird observatories during the spring migration: Lågskär Island (N 59°50', E 19°55') between 22 and 25 May, and in Tauvo between 29 and 31 May. In addition, migratory bird samples were collected in Kokkola archipelago (N 63°52', E 23°04') on 30 July 2005.

Birds were captured with mist nets, and identified by bird ringers. Blood samples were obtained by absorbing blood into filter paper slips from the veins of wings or feet, and then dried. Native blood samples were also harvested into small glass capillaries when possible.

CELL LINES (**I**, **II**, **V**)

The Vero and Vero E6 cell lines (both African green monkey kidney epithelial cells) were originally obtained from the American Type Culture Collection, ATCC.

METHODS

VIRUS ISOLATION (**II**, **V**)

Virus isolation was attempted from human skin tissue and whole blood samples (in EDTA-tubes), and from a subset of the bird blood samples. The frozen skin samples were cut into small pieces, then homogenized in a mortar, and suspended in 100-150 µl Dulbecco's minimal essential medium (MEM) + 0.2% bovine serum albumin (BSA). Confluent Vero cells in 25-cm^2 cell culture flasks were inoculated

with 50-100 µl of this suspension diluted in 500 µl of culture medium, containing MEM and 2% fetal calf serum with a mixture of glutamine, ampicillin and penicillin. Whole blood samples were diluted 1:10 in culture medium and confluent Vero cell cultures were inoculated with the final volume of 500 µl. The cells were incubated for 1 h at 37°C, then 3-4 ml culture medium was added and the cultures were kept at 37°C.

The toxicity of EDTA-anticoagulant on Vero cells could be avoided by completely removing the blood dilution from cells after the 1-hour incubation, and also by changing the culture medium the following day after the inoculation, and then twice a week. All cell cultures were inspected daily. When CPE was apparent, immunofluorescence assay (IFA) was performed and the cells were passaged.

RNA EXTRACTION (**II**, **III**, **V**)

Viral RNA was extracted from virus culture supernatants and from skin tissue samples by use of TriPure Isolation Reagent (Roche Molecular Biochemicals), and from whole-blood samples by use of PAXgene Blood RNA Kit (PreAnalytiX). For each experiment, negative (water) and positive (RNA from SINV supernatant, always handled in a separate laboratory) controls were included.

NUCLEIC ACID DETECTION WITH RT-PCR (**II**, **III**, **V**)

Nested RT-PCR was developed for nucleic acid detection in the clinical samples and bird blood samples. The sequences of primers (within the E2 region) designed for the nested RT-PCR were as follows: 5′-ATACGAC(C/A)AAAGCGGAGCAG-3′ (outer forward [OF]), 5′AGTACGGGTCGTAACGGTTC-3′ (outer reverse [OR]), 5′-GATACTTTCTCCTCGCGAAATG-3′ (inner forward [IF]), and 5′-GTCTTGTAATCGCCGCACTTG-3′ (inner reverse [IR]).

For the RT reaction, RNA was incubated with 20 pmol of each outer primer, 40 U of M-MuLV reverse transcriptase (MBI Fermentas), 5 pmol (0.22 mmol/L) of dNTP mix (Finnzymes), and 80 U of ribonuclease inhibitor (MBI Fermentas) in RT buffer (MBI Fermentas), in a total volume of 22.5 mL, for 90 min at 37°C.

For the outer PCR, 10 mL of complementary deoxyribonucleic acid (cDNA) was incubated at 95°C for 5 min, cooled immediately on ice, and mixed with 32 pmol

of OF, 24 pmol of OR, 20 pmol (0.2 mmol/L) of dNTP mix (Finnzymes), and 2.5 U of Taq DNA polymerase in PCR buffer with 200 mmol/L $(NH_4)_2SO_4$ and 2.5 mmol/L MgCl2 (MBI Fermentas), in a total volume of 100 mL. The PCR conditions were 39 cycles of 96°C for 45 s, 53°C for 45 s, and 72°C for 40 s; followed by a final extension for 10 min at 72°C.

For the inner PCR, 5 mL of the outer PCR product was mixed with 28 pmol of each inner primer, 25 pmol (0.25 mmol/L) of dNTP mix (Finnzymes), and 2 U of Taq DNA polymerase (MBI Fermentas), as described above. The PCR conditions were 39 cycles of 95°C for 60 s, 56°C for 45 s, and 72°C for 30 s; followed by a final extension for 10 min at 72°C.

The products were stained with ethidium bromide and run in SeaKem LE agarose (BioWhittaker Molecular Applications). The sensitivity of the PCR assay was determined to be ~0.1 infectious virions, by use of SINV supernatant (titrated with plaque titration) as a control.

CLONING, SEQUENCE ANALYSIS AND PHYLOGENY (**II**)

Phylogenetic analysis was performed within the nucleotide sequences of 1,178–1,281 bp from nsP3 and nsP4 region (nucleotides 5,258-6,510; the genome position is given according to the published sequence of the strain AR339 (HRsp variant) [227]). The PCR amplicons were purified with QIAquick gel extraction kit (Qiagen). The amplicons were cloned with TOPO TA Cloning Kit for Sequencing (Invitrogen), following the manufacturer's instructions, and transformed into TOP10 chemically competent *E. coli* cells on bacterial plates containing x-gal (5-bromo-4-chloro-3-indolyl-ß-D-galactoside) and IPTG (isopropylthio-ß-D-galactoside) for blue-white screening. The plasmid DNA was isolated with QIAprep miniprep kit (Qiagen) and restriction analysis was performed. Vector-based primers M13 Reverse and T7 were used for automatic sequencing with ABI PRISM.

Sequences were aligned with Clustal W 1.75 program [235] into MSF-format and edited with GeneDoc multiple sequence alignment editor program. Sequence was confirmed from at least three different clones. PHYLIP program package (Felsenstein, 1993) was used to create 5000 bootstrap replicates on the sequence data (SEQBOOT). Distance matrices were calculated with DNADIST program

with Kimura's two-parameter model of substitutions and analyzed by Neighbor-joining tree-fitting algorithm with NEIGHBOR program. The bootstrap support values were calculated with CONSENSE program.

SINV IGM AND IGG EIAS (**I**, **II**, **III**, **IV**, **V**)

SINV IgM and IgG EIA methods were developed and evaluated as a part of this study (**I**).

Virus production for the EIAs

SINV (Edsbyn 82/5 [181]) was propagated in Vero cells in MEM, 0.2% BSA, glutamine-penicillin-streptomycin. Cell culture supernatants were collected at 50% cytopathic effect, and centrifuged in Sorvall GSA rotor for 15 min at 1000 x g at +4°C. The resulting supernatant was filtrated (0.22 μm) and ultracentrifuged in Beckman SW 28 rotor for 3.5 h at 122,000 x g at +4°C. The pellet was eluted overnight in 300 μl of 50 mM Tris-HCl and 1 mM EDTA pH 7.5 at +4°C, water-sonicated for 5 min on ice, and used as EIA antigen. To confirm quality, the virus was examined with electron microscopy.

Coating of the EIA plates

The antigen was inactivated with 10% N-octyl-β-D-glucopyranoside (20:1) for 15 min on ice, and water-sonicated for 8 min on ice. Box titration was performed to find out the optimal antigen dilution, which was 1:2500 in phosphate-buffered saline (PBS). After coating with 100 μl of antigen dilution at room temperature overnight, 96-well-plates were washed five times with PBS + 0.05% Tween 20, and blocked with 100 μl of 3% BSA in PBS for 1 h at room temperature. The plates were once washed, and stored at -70°C.

IgM and IgG EIAs

Diluted sera (1:200 in PBS + 0.05% Tween 20 + 0.5% BSA) were incubated for 60 min at +37°C on antigen-coated wells, washed 5 times with PBS + 0.05% Tween 20, again incubated for 60 min at +37°C with 100 μl of horseradish peroxidase labeled rabbit-anti-human IgM (1:1600) or IgG (1:4000) (DakoCytomation), and washed. The substrate reaction with 100 μl of 3,3',5,5'-tetramethyl benzidine (Sigma-

Aldrich) was stopped with 100 µl of 0.2M H_2SO_4 after 10 min, and the optical density (OD) values were read within 30 min by a spectrophotometer at 450 nm.

ELECTRON MICROSCOPY (**I**)

Propagated and purified virus was applied on carbon-coated grids and negatively stained with potassium phosphotungstate (2%, pH 7.2). The grids were examined with Jeol JEM-100 CXII electron microscope.

HI TEST (**I**, **II**, **III**, **IV**, **V**)

HI test was used as the primary screening method for bird blood filter paper solutions, and as a specificity control and for evaluation of the EIAs. The method was modified from Clarke and Casals (1958) [46]. Sera diluted 1:5 in borate pH 9.0 + 0.6% BSA were adsorbed with an equal volume of 25% kaolin (in borate pH 9.0), shaken for 20 min at room temperature, and centrifuged for 20 min at 700xg at +4°C. Erythrocytes were separated from male goose blood, and washed with dextrose-gelatin-veronal buffer and penicillin-streptomycin. The samples (500 µl) were adsorbed with 50 µl of 50% erythrocytes (in dextrose-gelatin-veronal buffer and penicillin-streptomycin) at +4°C for 20 min, while lightly shaken every 5 min. After centrifugation for 10−15 min at 400 x g at +4°C, the resulting supernatant was separated.

Pre-treated samples underwent two-fold microtitration on 96-well-plates (25 µl per well; diluted in borate pH 9.0 + 0.6% BSA), and were incubated with 3 hemagglutinating units (25 µl) of Tween-ether-treated SINV supernatant (strain AR339 [232], grown in Vero cells) overnight at +4°C. Tween-ether-treatment was done under a hood on ice and inactivation of the antigen was confirmed on Vero cell cultures. Separate microtitration confirmed correct antigen concentration. Goose erythrocytes (1:10 in dextrose-gelatin-veronal buffer + penicillin-streptomycin) were further diluted with 0.20 M NaH_2PO_4 and 0.20 M Na_2HPO_4 (both in 0.15 M NaCl) to a final 0.2% suspension (pH 6.0), of which 50 µl was added on each well, and kept at room temperature. The HI result was read 1 h later.

NT (**I**, **V**)

NT was used as a confirmatory method for EIA and/or HI with human sera or bird blood filter paper slip solutions. Endpoint titers of neutralizing antibodies against SINV-prototype strain AR339 were determined by detecting inhibition of CPE on confluent monolayers of Vero E6 cells. The sera or bird blood filter paper slip solutions were diluted in culture medium containing MEM and 2% fetal calf serum with a mixture of glutamine, ampicillin and penicillin. 300 µl (containing approximately 50 plaque forming units) of virus dilution was mixed with an equal volume of serial fourfold serum dilutions, followed by incubation for 1 h at +37°C. Vero E6 cells were subsequently inoculated with 200 µl of this mixture, and incubated for 1 h at +37°C. 1.5 ml culture medium was added on each well. The endpoint titers in which CPE was inhibited were determined approximately 65 h post infection.

IFA (**I**, **II**)

An IFA was developed to confirm SINV infection of the cells in virus isolation experiments (**II**), or conversely, to detect SINV antibodies in serum samples (**I**). Vero cells (from virus isolation experiments or inoculated with SINV) were harvested (in 600 µl PBS in virus isolation experiments), washed and centrifuged at 1800 rpm for 3 min x 5 and dried on a slide. For immunofluorescence staining the slides were fixed for 7 min in ice-cold acetone. A pool of 10 SINV IgG-positive sera or the test serum was diluted 1:20 in PBS, added to slides and incubated in a moist chamber at 37°C for 30 min. The slides were washed three times in PBS and once in aqua, then incubated at 37°C for 30 min with fluorescein isothiocyanate-conjugated F(ab')2 goat anti-human IgG diluted 1:100 in PBS. After another wash, the slides were dried, mounted and screened with a fluorescence microscope.

IMMUNOBLOTTING (**I**)

Immunoblotting was used to characterize the antigenic proteins of the SINV EIA antigen. Non-reduced (dissolved in Laemmli sample buffer) and reduced (dissolved in Laemmli sample buffer with 2-mercaptoethanol (10 w/v %)) SINV preparates were separated by 10% and 8-16% non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Coomassie staining was

performed to reveal protein bands. The SDS-PAGEs were transferred into nitrocellulose filters and immunoblotted with sera (diluted 1:50) according to standard protocols, using polyclonal rabbit anti-human IgM or IgG (DakoCytomation). The reaction was detected by enhanced chemiluminescence [194].

IMMUNOHISTOCHEMICAL ANALYSIS (**III**)

Immunohistochemical analysis was used for skin tissue samples. The skin biopsy specimens were fixed in 10% formalin and embedded in paraffin blocks. The immunohistochemistry assay was performed on two thin sections cut from each block and mounted on Super Frost (+) slides (Menzel-Gläser). Ventana Discovery (Ventana Medical Systems) was used for DAB (3,3' diaminobenzidine) staining. The tissue sections were deparaffinized, and incubated for 20 min with a mixture of three different sera of mice infected with SINV (two with the strain Ockelbo Edsbyn 82/5 and one with Ockelbo 520; kindly provided by Sirkka Vene, Swedish Institute for Infectious Diseases Control) in 1:1500 dilution. The samples were counterstained with hematoxylin and evaluated with light microscopy. A skin biopsy specimen from a serologically confirmed SINV–negative patient was used as a negative control sample. A positive control sample was created from SINV-infected Vero cells (dispersed in 4% agarose) that were formalin-fixed and paraffin-embedded.

RESULTS AND DISCUSSION

The results of this study, based on the original contributions (**I**-**V**) and unpublished data, are summarized below. The study describes serodiagnostic methods for human SINV infection, the etiologic agent of Pogosta disease, clinical and laboratory manifestations of the disease, and epidemiology of SINV infection in Finland.

1. SERODIAGNOSIS OF SINV INFECTION (I)

The EIA methods described below were used for the serodiagnostics of the patients throughout the study.

EIA FOR THE SERODIAGNOSTICS OF SINV INFECTION

During the Pogosta disease epidemics in Finland, thousands of serum samples are sent to the laboratory within a few months time for SINV antibody testing. Due to requirements on time- and cost-effectiveness, the previously published methods [40,242] were not compatible for our diagnostic purposes. Therefore, we set up IgM and IgG EIAs based on highly purified SINV, and evaluated the methods by comparison to HI, IFA, and NT.

Assay cut-offs were determined with 40 confirmed negative samples (**Figure 6**). The EIAs were evaluated with paired serum samples from 46 patients (**I**: Fig. 1), who all showed neutralizing antibodies in either sample, and at least a four-fold rise in HI titers between the samples (**I**: Table 1). The sum of IgM and IgG OD values compared to HI titers correlated significantly, Pearson's r = 0.406, significance level (one-tailed) = 0.001 (only HI-positive samples included, N=62) (**I**: Fig. 2).

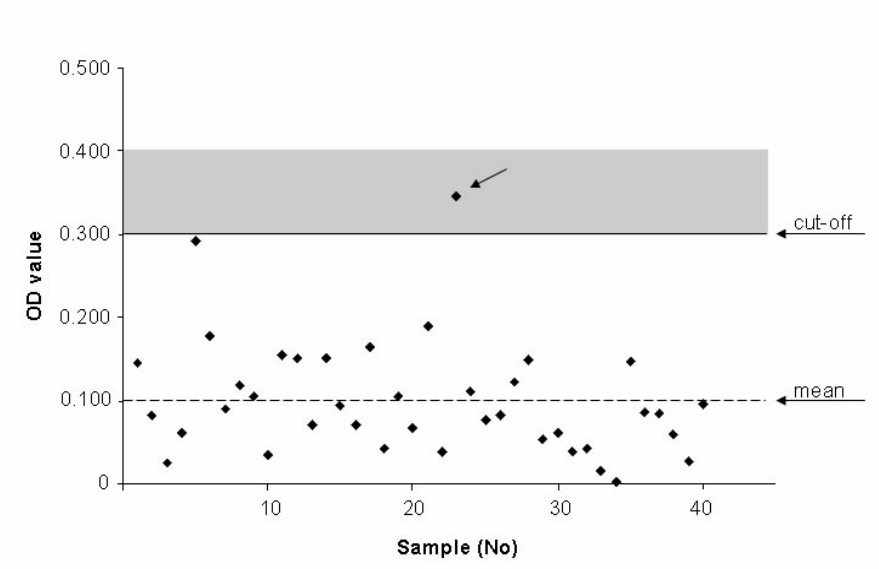
The sensitivity of the IgG EIA was 100% (46/46 positive), and IgM EIA 97.8% (45/46 positive; the negative sample was, however, taken 26 days after onset of symptoms), as determined with the second samples from the positive panel. All the second samples were positive (titer ≥20) also in the IgG IFA analysis. The specificity of the IgG EIA was 97.6% (1/42 false grey zone) and IgM EIA 95.2% (2/42 false-positive), determined with an independent negative panel.

The EIAs developed where shown to meet the desired requirements: the antigen production is fairly simple, but at the same time, the methods are adequately sensitive, and specific, especially when HI and EIA are performed in parallel. However, in the future, development of EIAs based on e.g. μ-capture format could further improve the reliability of the serodiagnosis of SINV infection.

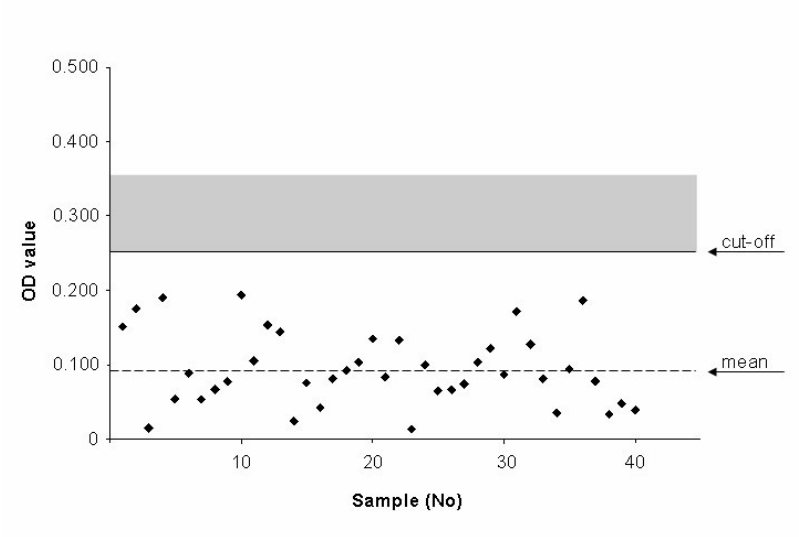
Figure 6.

40 serum samples from patients negative for SINV HI antibodies were twice analysed with SINV IgM and IgG EIA. Each dot represents the mean value of two analyses, subtracted with the mean value of eight blank wells. Assay cut-off was determined by calculating mean plus 3 SD. Grey zone determined for the assay is shown in grey. 1/40 samples gave a borderline result (arrow), while all others remained negative A. IgM EIA, B. IgG EIA. OD, optical density.

A



B



ANTIGENIC PROPERTIES OF THE PURIFIED SINV USED AS EIA ANTIGEN

The described EIAs are based on highly purified SINV grown in Vero cell cultures. To characterize the antigenic proteins of the EIAs, the antigens were separated with SDS-PAGE (**I**: Fig. 3) and immunoblotted (**I**: Fig. 4). Convalescent serum samples of patients with SINV infection had strong reactivity against both E1 and E2 in IgM and IgG immunoblots. Also in one of the first samples (46I), IgM reacted with E2, and faintly with E1. A strong IgM response was seen against C protein in sample 46II**;** however, C protein showed background signal in immunoblot in all samples, including the negative sera. Thus, both E1 and E2 appear to contribute to the antigenicity in the IgM and IgG EIAs described. The antigenic role of C protein in early infection remained unclear. According to literature, SINV E1 is responsible for hemagglutinating activity of the virus, whereas neutralizing activity is targeted against E2 [208]. It is evident that the majority of the neutralizing activity is due to IgM response in the early samples, whereas in the late samples the activity is largely due to IgG, which could explain the lack of differences in NT titers in some individuals in our study.

DIAGNOSTIC CRITERIA

The risk for false-positive diagnosis of acute disease using the IgM EIA described is less than 4.8%, because of further verification of the specificity of the reaction by HI and the following diagnostic criteria for acute SINV infection: (1) in addition to a positive IgM result, a ≥4-fold increase in HI (or IgG) titer between paired sera or (2) a positive IgM result and a negative/borderline IgG result (which excludes old immunity) and specific reaction in HI (titer >10). A positive IgG and HI result confirm previous immunity.

IgM antibodies to alphaviruses are complex-specific [37]. The EIAs described here could be cross-reactive with other alphaviruses. This is not a considerable problem in Northern Europe, since besides SINV, no other alphaviruses are known to circulate in the region. However, travelers' imported infections, e.g. CHIKV, are an exception.

2. ETIOLOGY OF POGOSTA DISEASE (II)

Prior to the present study, SINV had been isolated from a human sample twice: from the vesicle fluid of skin lesions taken from a 45-year-old woman with acute rash-arthritis in South Africa in 1963 (Girdwood strain) [164], and from the serum sample of a febrile patient in China in 1992 (YN87448 strain) [263]. Although it has been generally accepted that SINV or a virus closely related to SINV is the causative agent of the rash-arthritis syndrome, Pogosta disease, in Finland, the association was thus far only based on serological evidence [28,31].

We confirmed the etiology of Pogosta disease by isolating SINV from five acutely ill patients with serologically confirmed SINV infection (**II**: Fig. 1, Table). The isolates were confirmed positive with IFA, nested RT-PCR, and subsequent sequence analysis. Of the isolates four were recovered from a skin tissue biopsy specimen, and one from a whole blood sample. These strains, representing the first SINV isolates from Finland, confirmed that SINV is the etiological agent of Pogosta disease, and are the first human SINV isolates from Europe.

3. CLINICAL PICTURE OF ACUTE POGOSTA DISEASE (III)

Two previous studies have outlined the most typical symptoms of acute Pogosta [239] and Ockelbo [76] disease as arthritis, rash, and fever. To further characterize the clinical picture of the acute infection, we studied 86 patients with serologically verified acute SINV infection in North Karelia during the 2002 Pogosta disease outbreak. See **Figure 5** for the material available for this study.

The clinical picture (**III**: Table 2) of the acute SINV infection consisted of joint symptoms, itching rash (**III**: Fig. 1), fatigue, mild fever, headache and muscle pain, usually beginning within the first two days of illness (**III:** Table 2). Ankle, finger, wrist and knee joints were most commonly affected (**III**: Fig. 2), and usually three or more joints were ill, manifesting with tenderness in movement and ache, but commonly also with edema (**III**: Table 3). Typically the joint symptoms occurred in a fluctuating manner, but not changing the anatomical location. There was no considerable difference in symptoms according to the time of the day (**III**: Table 3). The rash was usually located in trunk and thighs (**III**: Fig. 2).

In one of the patients from whom the clinical timeline was well documented the incubation period was probably 8-9 days (**III:** Fig. 3). However, further evidence is required to establish the incubation period for SINV infection. For comparison, in RRV infection, the incubation period is 7-9 days [89]. Most patients considered the subjective handicap and need for daily adjustments in their everyday lives during the symptomatic phase of the infection mild or moderate, but more than one third graded the subjective handicap considerable or major (**III**: Table 4).

Clinical hematological and infection laboratory parameters were analyzed at the acute phase. All parameters studied were within normal range in almost all cases (**Table 2**). In addition to the 86 patients with verified SINV infection, 28 seronegative and 6 old-immunity patients were initially recruited to the study. In comparing the patients with and without acute infection, it became evident that arthritis and rash were only somewhat less frequent in the latter. Thus, diagnosis of acute SINV infection should always be verified serologically; clinical diagnosis alone is not reliable.

Table 2.

Clinical hematological findings from patients who had acute SINV infection. Patients under the age of 17 have been excluded from this table due to different normal range values. F, female; M, male; WBC, white blood cell count; RBC, red blood cell count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

	MEAN (F/M)	MEDIAN (F/M)	RANGE (F/M)	N (F/M)	Normal range (F/M)
WBC, E9/L	**5.7**	5.3	1.9-10.7	43	3.6-10.1
RBC, E12/L	**4.4/5.0**	4.5/5.1	3.4-5.2/4.2-5.4	32/8	4.0-5.3 / 4.5-6.1
Hemoglobin, g/L	**133/155**	133/155	105-149/128-170	34/9	125-160 / 135-180
Hematocrit, %	**39/44**	39/46	33-44/37-47	33/9	37-47 / 40-54
MCV, fL	**88**	89	72-104	42	80-96
MCH, pg/cell	**30**	31	24-37	42	27-32
MCHC, g/L	**344**	343	320-366	40	320-360
Platelet count, E9/L	**228**	218	144-373	41	150-400
Lymphocyte %	**33.1**	32.2	15.2-53.2	30	20-45
Neutrophil %	**56.5**	60.0	35.5-79.0	27	40-75
CRP, mg/L	**-**	-	<5-20	15	<10
ESR, mm/h	**12/3**	14/3	2-22/3-3	10/1	1-10 / 1-8

4. PROGNOSIS OF POGOSTA DISEASE (III, IV)

PERSISTENCE OF JOINT MANIFESTATIONS

Several reports have suggested that joint symptoms in arthritogenic alphavirus infections can persist for months or even years, but most of these studies have been carried out with self-administered interviews without examining the patients [17,50,178,180,182,218,242]. In a single previous clinical study on SINV infection performed by Laine *et al.* with 26 patients 2.5 years after serologically confirmed SINV infection, prolonged joint manifestations were present in 42% of the patients [133].

To further shed light into the possible prolonged manifestations of Pogosta disease, we followed our patients, recruited to the study during the 2002 outbreak, first by means of self-administered questionnaires at 5-6 months and 12-13 months post infection, and then by physical examination and interview at three years post infection.

In the 12-13-month follow-up questionnaire study (**III**), 50% of the patients reported that joint symptoms were still present at 12 months post infection (**III**: Table 3). The duration of joint symptoms was significantly dependent on age: the average age for those with symptoms for more than a year was 46 years, whereas it was 35 for those with full recovery (Student t-test, $p = 0.014$). Although females manifested with prolonged symptoms more often than men, the difference was not significant (Chi-Square test, $p = 0.0729$).

The proportion of patients reporting prolonged joint manifestations was surprisingly high. However, the methodology used (self-administered questionnaires) is susceptible to errors of recall or other mistakes, bringing on limitations in the interpretation of the results. Therefore, another, more reliable approach was undertaken: we physically examined the same patients three years after the infection to reveal objective joint manifestations that could be linked with SINV infection (**IV**). The rheumatologic assessment was performed by a senior rheumatologist, and the general examination by a general physician. In all, 49 patients could be recruited at this stage (60% of the 81 that could be invited) (**IV**: Table 1).

The joint examination revealed several findings according to which the patients were categorized into groups A-D (**IV**: Table 2). Arthritis, which was determined as swelling and tenderness in the joint in the physician's examination, was diagnosed in 2/49 (4.1%) of the patients (Group A). Tenderness in palpation or in joint movement was found in 14.3% of the patients in the rheumatologic examination (Group B), whereas additional 10.2% of the patients complained of arthralgia at the interview (Group C); thus a total of 24.5% of the patients had such joint symptoms that could be linked with the SINV infection three years earlier (**IV**: Table 3). Patients in Group D (75.5%) had no clearly defined joint symptoms that could be associated with SINV infection.

The prevalence of persistent joint pain caused by SINV infection shown in this study is in agreement with previous studies. However, our results suggest that persistent arthritis, requiring both swelling and tenderness in examination, is not a typical manifestation, and should be distinguished from mere pain.

It can be estimated that solely in the province of North Karelia, which is a highly endemic area, and where this study was carried out, several hundred patients

manifest with chronic arthritis, and a few thousand people from arthralgia as sequelae of SINV infection. Consequences and clinical significance of Pogosta disease could be considerable, and it is important to consider prolonged symptoms of SINV infection as a possible cause of persistent and undefined joint symptoms in areas with high seroprevalence.

The persistent disease mostly affected small and peripheral joints. The number of (subjectively) affected joints of the patients in the groups A-C increased in 4/12, remained the same in 2/12, and decreased in 6/12 of the patients as compared to the acute phase; 69% of the affected joints were ill also during the acute phase. Frequency of persistent joint symptoms could not be associated with gender or age. None of the patients in groups A-C fulfilled the criteria of spondyloarthropathy.

One of the patients had been diagnosed with Sjögren's syndrome and one with polymyalgia rheumatica during the three-year follow-up. Whether SINV infection can initiate autoimmune diseases remains a subject for further investigation. There was no significant difference in the history of other musculoskeletal diseases between patients in group A-C and group D.

Our study arrangement differed from the clinical study performed by Laine *et al*. at the end of 1990s [133]. The patients in that study were examined by a general physician, and those patients having joint symptoms were examined by a rheumatologist. In the present study all patients were examined by a rheumatologist. In our study, general examination was performed on each patient by a general physician, and we had records of each patient's acute phase (questionnaire forms fulfilled during the acute phase), thus our study was prospective. This allowed us to compare the number of joints affected at the acute phase and three years post infection. We were also able to compare whether the same joints were affected at these two stages. We classified our patients according to the severity of their symptoms. We took into account whether the findings were subjective or objective, as well as the temporal association of the symptoms to the infection three years earlier, and report the exact joints that were affected (number of patients, number of joints, anatomic location), not available in previous studies.

Studies reporting persistent joint manifestations after an alphavirus infection are summarized in **Table 3.** Our results are in agreement with the previous reports on

SINV, as well as on RRV. Comparison to CHIKV, BFV, MAYV, and ONNV is difficult, as only few studies have been performed, and only with small number of patients. Conclusion from our studies and those by Laine *et al*. [133] is that Pogosta disease causes persistent joint manifestations, sometimes even chronic arthritis, but our data indicate that persistent arthritis continuing for years is not a very typical manifestation.

Table 3.

Summary of studies reporting persistence of joint symptoms after an alphavirus infection.

Virus	No. of patients	Time post infection (months)	% of patients with residual joint manifestations	Study setting	Additional information	Reference
SINV	86	3-4 years	24*	questionnaire and telephone interview	* 67% of these were symptomatic 5-6 years post infection	Niklasson and Espmark 1986, Niklasson *et al* 1988
SINV	16	2.5 years	31	telephone interview		Vene *et al* 1994
SINV	60	> 1 month	55	self-administered questionnaire		Turunen *et al* 1998
SINV	26	2.5 years	42	clinical examination	8% had arthritis	Laine *et al* 2000
SINV	1	> 4 years		clinical examination	the patients had osteoarthritis	Luukkainen *et al* 2002
SINV	64	> 12 months	50	self-administered questionnaire		**III**
SINV	49	3 years	25	clinical examination	4% had arthritis	**IV**
RRV	255	24-42 months	57	self-administered questionnaire		Condon and Rouse 1995
RRV	436	13 months	12	self-administered questionnaire	3% reported swollen joints	Selden and Cameron 1996
RRV	47	2.3-6.5 months	68	standard questionnaire		Harley *et al* 2002
CHIKV	28	20 months	18	clinical examination		Fourie and Morrison 1979
CHIKV	20	4-16 months	60-70	clinical examination		Kennedy *et al* 1980
CHIKV	1			clinical examination	destructive arthropathy for 15 years	Brighton and Simson 1984
BFV	53	>6 months	50	self-administered retrospective questionnaire		Beard *et al* 1997
BFV	?			?	~10% have joint tenderness at 6 months post infestion, unpublished data reported in this review	Flexman *et al* 1998
MAYV	2			subjective report	a few months of joint pain after infection	Tesh *et al* 1999
MAYV	1			clinical examination	recurrent arthralgias	Taylor *et al* 2005
ONNV	?			?	"protracted" disease "in some patients"	Shore 1961

PROGNOSIS OF EXTRA-ARTICULAR MANIFESTATIONS

According to the questionnaire study carried out with the Pogosta disease patients, defervescence typically occurred within a few days, but rash and fatigue often lasted for more than 5 days (**III**: Table 2). At three years after infection, the patients (n=49) were physically examined by a physician to reveal any extra-articular manifestations. The examination included cardiac and pulmonary auscultation, abdominal palpation, examination of skin and mouth, and palpation of lymph node areas. None of the patients had such extra-articular findings at three years post infection that could be linked with SINV infection (**IV**), consistent with previous reports.

5. ANTIBODY RESPONSE AND PRESENCE OF VIRUS IN TISSUES DURING HUMAN SINV INFECTION (II, III, IV)

ANTIBODY KINETICS (**III**, **IV**)

To facilitate the interpretation of SINV serodiagnostics, we aimed to characterize the antibody kinetics in SINV infection. Serial serum samples of 50 patients taken during 0-6 months post infection were analyzed for the presence of IgM and IgG antibodies (**III**: Fig. 4a-f). IgM antibodies became detectable within the first 8 days of illness, and 36% of the patients had IgM antibodies detectable 5-6 months post infection (two of them were positive in rheumatoid factor testing). In the first serum samples, 48% of the 86 patients had a positive IgM result, and 16% of the patients had a borderline result. IgG antibodies became detectable within the first 11 days of illness. To conclude, a negative result in SINV antibody testing during the first week of illness does not rule out Pogosta disease, and a paired sample is required.

A serum sample was retrieved from 44 patients at three years post infection (**IV**). SINV IgM antibodies were detectable (after removal of IgG antibodies) in 3/44 (6.8%) of the samples, and a borderline IgM result was detected in an additional 3/44 (6.8%) of the samples. Persisting IgM antibodies complicates the distinction between acute infection and previous immunity; the problem could be solved by developing an appropriate IgG-avidity test in the future.

Both patients with arthritis had IgM antibodies detectable in their serum samples at three years post infection (**IV**: Table 3). Although IgM antibodies have previously been shown to persist even for several years post infection, no correlation between persisting IgM and prolonged joint symptoms has been stated [182]. Due to limited number of samples, we could not show statistical significance either. Persisting IgM antibodies could suggest that viral replication takes place somewhere in the body, possibly in synovia, periarticular tissues, muscle cells, or elsewhere.

DETECTION OF SINV IN BLOOD AND SKIN (**II**, **III**)

Previously, SINV RNA has been detected by PCR in skin lesions of Swedish Ockelbo disease patients [118]. We collected both skin lesion biopsy specimens and whole blood samples from acutely ill patients with serologically verified SINV infection. Virus isolation was successful from 4/23 skin lesion samples and from 1/73 whole blood samples (**II**). SINV RNA could be detected by RT-PCR in 4/23 skin lesions, and in 5/73 whole blood samples (**III**). Altogether, SINV could be isolated or detected by RT-PCR (or both) from 8/86 patients (**Table 4**). These samples were taken during the first 2-9 days of illness (data available from 6/8 cases). No SINV antigen was detected in the 21/23 of the skin biopsies analyzed by immunohistochemistry. Two of the skin lesion specimens were negative with RT-PCR, although virus isolation was successful. This could be due to a varying viral load in different sections of the skin, possibly related to the amount of blood in the skin tissue.

The cell type in which SINV replicates in skin tissue is not known. Histopathologic examination of skin lesions of Pogosta disease patients has previously revealed a pronounced lymphohistiocytic inflammatory infiltrate and lymphoblast-like cells [11]. Since the virus is present in the skin during acute infection before the onset of antibody response (**II**: Table), the cutaneous manifestations in Pogosta disease may be due to a direct viral effect – or the pathogenesis could involve a more complex immunologic reaction.

Table 4.

Serological findings and detection of SINV from skin lesion biopsy specimens and whole blood samples taken from acute patients who had acute SINV infection. The 8 patients from whom SINV RNA could be detected or virus isolation was successful (or both) are presented separately (P1-P8). See **III**: Fig. 3 for the clinical timeline of the patient 5 (P5) and **III**: Fig. 1 for the photograph of the rash of patient 7 (P7). N/A, sample not available. *IgM status not known at the time of sampling the skin lesion, since serum sample was taken 9 days before that.

		All		Individual patients							
		N	%	P1	P2	P3	P4	P5	P6	P7	P8
SINV IgM status in first serum sample	Negative	31/86	**36**	**x**	**x**		**x**	**x***			**x**
	Borderline	14/86	**16**			**x**			**x**	**x**	
	Positive	41/86	**48**								
SINV RNA detectable in blood		5/73	**7**	**+**	**+**	**+**	**-**	**+**	**-**	**-**	**+**
Virus isolation from blood		1/73	**1.4**	**-**	**-**	**+**	**-**	**-**	**-**	**-**	**-**
SINV RNA detectable in skin		4/23	**4**	**+**	**-**	**N/A**	**-**	**+**	**+**	**+**	**-**
Virus isolation from skin		4/23	**17**	**+**	**+**	**N/A**	**+**	**+**	**-**	**-**	**-**
Immunohistochemistry on skin		0/21	**0**	**-**	**-**	**N/A**	**-**	**-**	**-**	**-**	**-**

6. EPIDEMIOLOGY OF SINV IN FINLAND (II, V)

MOLECULAR EPIDEMIOLOGY OF SINV STRAINS FROM FINLAND (**II**)

The five new SINV strains that we isolated from acutely ill patients in Finland in 2002 were further characterized by phylogenetic analysis of the nucleotide sequences of 1,178–1,281 bp from nsP3 and nsP4 region, nucleotides 5,258-6,510; the genome position is given according to the published sequence of the strain AR339 (HRsp variant) [227]. The phylogenetic tree was constructed by using neighbor-joining tree-fitting algorithms. The strains were given the following accession numbers: Ilomantsi-2002A (AY532322), Ilomantsi-2002B (AY532326), Ilomantsi-2002C (AY532324), Kiihtelysvaara-2002 (AY532325), and Johannes-2002 (AY532323). In addition, we sequenced from this region the LEIV-9298-strain (AY532321) [159], isolated from Aedes mosquitoes in 1983 in central Russian Karelia, approximately 200 km north of Ilomantsi, Finland.

The following sequences available in GenBank were included in the comparison: AR339 (HRsp variant), Girdwood S.A., MRE16, Ockelbo (Edsbyn 82), S.A.AR86, SW6562, YN87448, and XJ-160 (**II**: Fig. 1). Sequence comparisons (**Table 5**) and phylogenetic analysis (**II**: Fig. 2) showed that Finnish SINV strains are closely

related to each other and to the Swedish and Russian strains, isolated from mosquitoes two decades earlier; these strains seem to share a common ancestor. The percentage difference of these strains was 0.1% to 1.4% on nucleotides, and 0% to 2.1% on amino acids. The Russian Karelian LEIV-9298 and Johannes differ by one nucleotide, and their amino acid sequences are identical in the analyzed region.

This analysis could indicate that Northern European SINV strains are maintained locally in disease-endemic regions. However, South African SINV strains do not differ substantially from Swedish strains [166] or from the Finnish strains in the present study. The question remains whether the yet unknown resident reservoir species are able to sustain SINV cycle in Northern Europe endemically or whether it is crucial that the virus is repeatedly introduced there from the southern hemisphere. Migratory birds would be plausible viral carriers from faraway distances, such as Africa.

INVOLVEMENT OF BIRDS IN THE EPIDEMIOLOGY OF SINV (**V**)

The phylogenetic studies on the novel Finnish SINV strains (**II**) and the data reported by Norder *et al*. [187] inspired us to look for factors contributing to the peculiar epidemiologic pattern of human SINV outbreaks every seven years in Finland. The observation that in the 1970s and the 1980s, the human outbreaks and crashes of the grouse population seemed to coincide (**Figure 7**), and the detection of SINV antibodies in Finnish [31] and Swedish [154] birds in the late 1980s, focused our interest on resident grouse.

Since the phylogenetic analyses also supported the hypothesis that SINV is redistributed in a longitudinal, not latitudinal, direction, studying migratory birds became of particular interest, as well. Indications of periodic redistribution of SINV strains over long distances and within a short time have previously been demonstrated in Australia [213], a finding consistent with the involvement of migratory birds. Furthermore, studies on antigenic relatedness of alphaviruses have also suggested that progenitor alphaviruses are spread over long distances by birds [39].

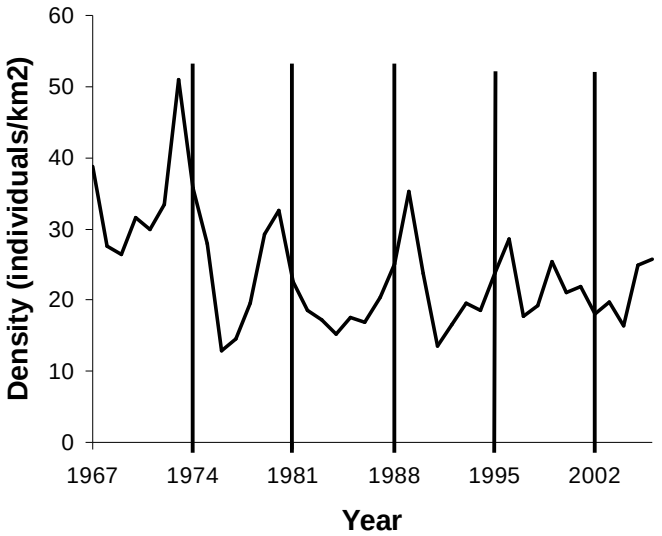
Table 5.

Nucleotide and amino acid differences of the analyzed SINV strains. Percentage differences of the strains on nucleotides (above the diagonal) and amino acids (below the diagonal).

	Ilomantsi-2002A	Ilomantsi-2002B	Ilomantsi-2002C	Kiihtelysvaara-2002	Johannes-2002	LEIV-9298	Ockelbo (Edsbyn 82)	AR339 (HRsp variant)	Girdwood S.A.	S.A.AR86	YN87448	XJ-160	MRE16	SW6562
Ilomantsi-2002A		0.6	0.6	0.3	0.4	0.5	1.4	8.3	5.2	7.4	5.0	26.5	35.8	33.3
Ilomantsi-2002B	0.5		0.3	0.2	0.2	0.2	1.2	8.0	5.0	7.2	4.8	26.4	35.6	33.4
Ilomantsi-2002C	0.7	0.7		0.2	0.2	0.3	1.1	8.0	5.0	7.0	4.8	26.4	35.6	33.3
Kiihtelysvaara-2002	0.2	0.5	0.7		0.1	0.2	1.1	8.0	4.9	7.1	4.7	26.3	35.6	33.3
Johannes-2002	0.2	0.2	0.5	0.2		0.1	1.0	7.9	4.8	7.0	4.7	26.3	35.6	33.3
LEIV-9298	0.2	0.2	0.5	0.2	0		1.1	8.0	4.9	7.1	4.7	26.5	35.6	33.4
Ockelbo (Edsbyn 82)	2.1	1.4	1.4	1.4	1.2	1.2		7.8	5.7	7.5	5.3	26.3	35.4	33.3
AR339 (HRsp variant)	6.4	6.4	6.4	6.7	6.2	6.2	6.5		7.8	10.0	7.5	26.1	34.3	32.4
Girdwood S.A.	3.3	3.3	3.6	3.6	3.1	3.1	5.0	6.7		4.4	2.2	26.8	36.3	34.1
S.A.AR86	6.9	6.9	6.9	7.1	6.7	6.7	6.5	8.9	6.2		3.5	28.3	37.2	34.4
YN87448	3.6	3.6	3.8	3.8	3.3	3.3	5.0	6.2	2.6	5.5		26.2	35.7	33.4
XJ-160	19.5	19.7	19.5	19.7	19.5	19.5	19.6	18.5	19.7	18.8	19.3		31.8	32.6
MRE16	28.3	28.5	28.3	28.5	28.3	28.3	28.5	27.4	28.0	27.7	27.9	23.6		32.6
SW6562	27.3	27.6	27.3	27.6	27.3	27.3	27.0	26.9	27.6	24.9	27.1	25.2	27.4	

Figure 7.

Annual late summer density estimates of grouse in the North Karelia game management district, based on route censuses in 1967-1988 and the wildlife triangle scheme in 1989-2006 (methods: Lindén and Rajala 1981 [145], Lindén *et al*. 1996 [144]; source: Finnish Game and Fisheries Research Institute). The vertical lines show the Pogosta disease outbreaks in Finland. Figure courtesy of Dr. Osmo Rätti.



*SINV antibodies in resident grouse (Tetraonidae) in Finland (**V**)*

In a previous study, SINV antibodies were found from 24/80 of game birds and 7/41 of mammals in Ilomantsi, Eastern Finland in 1981-1983 [31]. We collected 340 blood samples from resident grouse by hunters between 10 September and 31 October 2003, and 281 samples between 10 September and 31 October 2004 (**V**: Table 1, Fig. 1D). In 2003, one year after the human outbreak, the total prevalence of SINV HI antibodies in the grouse was 27.4%, but fell down to 1.4% in 2004 (**V**: Table 1). The difference was statistically significant ($\chi^2 = 76.8$, $p<0.001$).

The data indicate that grouse become exposed to SINV, and the virus could have an endemic cycle involving grouse. It remains unclear whether SINV can be pathogenic to grouse. Detection of high antibody titers in the grouse in 2003 implies that the birds have almost certainly been infected and produced a significant viremia. In 2003, the prevalence was very high in North Karelia (44%), Western Finland (Southern Ostrobothnia, Vaasa, and Central Ostrobothnia) (44%), and Central Finland (41%), but pronounced also in Lapland in Northern Finland (18%) (**V**: Fig. 1D) (**Figure 8**).

In 2003, 32.0% of the juveniles (born the same year) and 23.3% of the adults were seropositive, and in 2004, none of the juveniles and 2.9% of the adults were seropositive (96/621 of the grouse were of unknown age). As one third of the juvenile grouse were seropositive in 2003, it is likely that active transmission of the virus to the grouse population took place that year. In addition, a large proportion of these grouse showed a markedly high antibody titer. Capercaillie and Black Grouse showed the highest seroprevalence, both 31.3%; the rates for Hazel Grouse and Willow Grouse were 22.4% and 20.0%, respectively. The serological methods (HI and NT) that were used in this study are stringent and can only detect relatively potent antibody responses; the true seroprevalence of the birds may have been higher than reported here. In conclusion, resident grouse showed high seroprevalence of SINV one year after a human outbreak in Finland, and thus grouse might contribute to the human epidemiology of SINV. They could possibly act as amplifying hosts for SINV; however, longitudinal studies, and detection of the virus in grouse are needed to establish their role.

Figure 8.

Map of Finland and the hospital district division.

*SINV antibodies in migratory birds during spring migration (**V**)*

In a previous study, no neutralizing antibodies were found in the few hundred arriving migratory birds that were studied on the Swedish coast in 1983; from nesting birds (both residents and migrants), the antibody prevalence was 3.4% in July, and 10% in August of the same year [88]. In the UK, SINV antibodies have also been demonstrated in resident and migrant birds and poultry [32]. In Swedish passerine birds sampled in mid-summer during the 1990s (i.e. not during the spring migration), fieldfare (43.3%), redwing (37.0%), and song thrush (22.2%) each had a significantly higher SINV-antibody prevalence than the average (7.7%) of all species studied [153]. The prevalence was significantly higher in birds

sampled after the hatching year (13.9%), compared to the ones sampled during the hatching year (2.4%).

Prior to the present study, SINV antibodies had not been detected in migratory birds during their spring migration to Northern Europe. We collected 836 blood samples from migratory birds, of which 806 during spring migration in May 2004 and in May 2005 in Finland. SINV HI antibodies were detected in three individuals during spring migration: a robin (*Erithacus rubecula*) and a song thrush (*Turdus philomelos*) from Tauvo in 2004, and a red-backed shrike (*Lanius collurio*) from Lågskär in 2005 (**V**: Fig. 1A, Table 2). The song thrush was born the previous year; the age of the other two positive birds remained unknown. SINV RNA could not be detected with RT-PCR from these birds, and neither was virus isolation successful from the available whole blood samples of the seropositive robin and song thrush. This could be due to that detectable viremia had already passed, and studying seronegative birds instead could be worthwhile in the future. However, the low seroprevalence suggests that viable SINV in arriving migrants could be a rare event.

Robin and song thrush mainly winter in Western Europe (some individuals migrate to North-Western Africa), and red-backed shrike, instead, overwinters in eastern tropical and southern Africa [54]. Although the samples were collected in areas, which are usually the first landing areas for the birds when they arrive overseas, virus infection during the previous year in Northern Europe cannot be excluded. In conclusion, SINV-seropositive migratory birds arrive in Northern Europe during spring migration. However, detection of viable SINV in migratory birds remains to be shown, and would be the ultimate proof for their involvement in distributing SINV.

INCIDENCE AND SEROPREVALENCE OF SINV IN HUMAN POPULATION IN FINLAND (**V**)

We studied recent human epidemiology of SINV in Finland. A total of 2529 specimens submitted to the Helsinki University Central Hospital Laboratory during 1999-2003 were tested for SINV IgG-antibodies (IgM-positive samples were excluded). Standardized to the age distribution of the Finnish population, the estimated seroprevalence was 5.2% (**V**: Fig. 2). Brummer-Korvenkontio *et al*. have

previously reported that the seroprevalence of SINV in the Finnish population rose considerably from 1981 to 1995, and the seroprevalence in pregnant women in 1992 was 0.6% [31]; in our study, the seroprevalence in 20-39-year-old women was 2.3%, further suggesting a continuous increase in the seroprevalence of SINV in Finland.

In our material, seroprevalence in men was significantly (χ^2 = 4,721, p<0.030) higher than in women and the seroprevalence increased with age (**V**: Fig. 2), while the incidence rates for women were higher than for men. This unexpected finding might be explained by the possibility that infected men are more frequently asymptomatic than women, but more investigations are clearly required. The seroprevalence was high in Eastern Finland, especially in North Karelia and Kainuu, but also in Central Ostrobothnia in Western Finland (**V**: Fig. 1B) (**Figure 8**). Two previous studies in Sweden have reported that the most endemic area of SINV infections is between the 60th and 63rd parallels [76,155], corresponding to central Finland.

The SINV seroprevalence in 1850 hospital patients in Finland studied by Laine *et al.*, was 19% in individuals aged below 10 years [134]. Our results are in contradiction to this, as only 1.4% of the individuals aged 0-9 years were seropositive, and the seroprevalence increased gradually by age. In the age group 60-69 years 15% of the population had immunity to SINV. Considering the high infection rate, and that the infection may cause prolonged joint symptoms (**III, IV**) [133,182,239], the disease is a potential public health concern.

The incidence of SINV during epidemic years was 25.6/100,000/year in 1995 and 11.5/100,000/year in 2002 (**V**: Fig. 3); the average annualized incidence in the non-epidemic years (1996-2001 and 2003) was 2.4/100,000/year. In the study of Brummer-Korvenkontio *et al.*, the incidence in almost 10,000 Pogosta disease suspected individuals during 1980-1996 was 2.7/100,000 in Finland, being highest in the province of North Karelia and Central Finland [31]. In our study, rates were highest in North Karelia (25.7/100,000) (**V**: Fig. 1C); the incidence peaked in both North Karelia and Southern Ostrobothnia during the 1995 and 2002 outbreaks (**V**: Fig. 3). However, one year after the outbreak in 2003, the rates were twice as high in Southern Ostrobothnia compared with North Karelia (**V**: Fig. 3), possibly reflecting the high population immunity towards SINV in North Karelia after the

2002 outbreak. The high seroprevalence in Kainuu with a very low incidence could be due to considerable underdiagnosis.

Several unsolved questions still remain in the peculiar epidemiology of SINV in Northern Europe. In addition to birds, the seven-year cycle could involve other vertebrates, as well. On the other hand, the virus might not require a vertebrate host at all; it could be maintained through winter in transovarial mosquito transmission. Under experimental conditions, CHIKV is transmitted transovarially [265]; studies on SINV are not available. The limited time frame of clinical SINV infections in August and September is presumed to be due to that strictly late summer mosquito species function as vectors for the virus. A previous study suggests that the depth of snow cover in the late winter could predict the number of Pogosta disease cases [31]. *Culex* and *Culiseta* mosquito species spend early summer as larvae in aquatic milieu [27], which dry too soon, if the preceding winter has been poor with snow. This supports the idea that *Culex* and *Culiseta* mosquitoes, abundant in late summer, are important vectors for the virus.

CONCLUDING REMARKS AND PROSPECTS

Alphaviruses are found throughout the world, and some of them are causative agents of severe clinical diseases. Neuropathogenic alphaviruses are both equine and human pathogens, and their epidemiology, pathogenesis, and clinical course have been studied in detail. Despite the currently multifold incidence compared to encephalitic alphaviruses, research on alphavirus arthritides has been less active. This dissertation summarizes our studies on the etiology, clinical and laboratory manifestations, laboratory diagnosis, and epidemiology of SINV infection in Finland.

This study showed that SINV strains, closely related to other Nothern European strains and South-African strains, are the etiological agents of Pogosta disease. Our data suggest that resident grouse may be involved in the epidemiology of Pogosta disease, and migratory birds in distributing the virus over long distances.

Despite the seven-year cycle in the Pogosta disease epidemiology remains a mystery for the time being, the results warrant further investigations on birds. Longitudinal studies on resident grouse, and detection of viable virus in migratory birds would be required to establish their role.

We showed that after the acute SINV infection prolonged joint manifestations occur in a considerable proportion of patients, which may have public health implications, especially in the highly endemic areas. It can be estimated that solely in the province of North Karelia, where the seroprevalence is 10%, several hundred patients manifest with chronic arthritis, and a few thousand people with arthralgia as sequelae of previous SINV infection. SINV infection should be considered in the differential diagnosis of undefined, persistent joint symptoms.

Currently, the pathogenesis of the alphavirus arthropathy is poorly understood. Understanding the pathogenesis would be crucial for possible development of specific treatment or prevention for the infection, and therefore remains an interesting subject for future investigations.

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REFERENCES

1. Arboviral encephalitis cases reported in humans, by type, United States, 1964-2006. **Available at: http://www.cdc.gov/ncidod/dvbid/arbor/arbocase.htm**. Accessed July 23, 2007

2. National Infectious Disease Registry maintained by the Finnish National Public Health Institute (KTL Tartuntatautirekisteri). **Available at: http://www3.ktl.fi/stat/**. Accessed July 23, 2007

3. National Notifiable Diseases Surveillance System of Australia. **Available at: http://www9.health.gov.au**. Accessed July 23, 2007

4. Acheson NH, Tamm I. Replication of Semliki Forest virus: an electron microscopic study. **Virology 1967**;32:128-43.

5. Anderson BA. Focal neurologic signs in western equine encephalitis. **Can Med Assoc J 1984**;130:1019-21.

6. Anderson CR, Downs WG, Wattley GH, Ahin NW, Reese AA. Mayaro virus: a new human disease agent. II. Isolation from blood of patients in Trinidad, B.W.I. **Am J Trop Med Hyg 1957**;6:1012-6.

7. Anderson CR, Spence L, Downs WG, Aitken TH. Oropouche virus: a new human disease agent from Trinidad, West Indies. **Am J Trop Med Hyg 1961**;10:574-8.

8. Anderson SG, Caldwell NJ, Donneley M, French EL, Gee W, McLorinan H, MacDonald F, Robertson EG, Stevenson W, White J. Murray Valley encephalitis. **Trans Am Neurol Assoc 1951**;56:134-6.

9. Anishchenko M, Bowen RA, Paessler S, Austgen L, Greene IP, Weaver SC. Venezuelan encephalitis emergence mediated by a phylogenetically predicted viral mutation. **Proc Natl Acad Sci U S A 2006**;103:4994-9.

10. Aronson JF, Grieder FB, Davis NL, Charles PC, Knott T, Brown K, Johnston RE. A single-site mutant and revertants arising in vivo define early steps in the pathogenesis of Venezuelan equine encephalitis virus. **Virology 2000**;270:111-23.

11. Autio P, Niemi KM, Kariniemi AL. An eruption associated with alphavirus infection. **Br J Dermatol 1996**;135:320-3.

12. Ayres JC, Feemster RF. The sequelae of Eastern equine encephalomyelitis. **N Engl J Med 1949**;240:960-2.

13. Banatvala JE, Brown DW. Rubella. **Lancet 2004**;363:1127-37.

14. Barabe ND, Rayner GA, Christopher ME, Nagata LP, Wu JQ. Single-dose, fast-acting vaccine candidate against western equine encephalitis virus completely protects mice from intranasal challenge with different strains of the virus. **Vaccine 2007**;25:6271-6.

15. Bárdos V, Sluka F. Acute human infections caused by Tahyna virus. **Cas Lek Cesk 1963**;102:394-402.

16. Bárdos V, Sluka F, Cupková E. Serological Study on the Medical Importance of Calovo Virus. **In: Bárdos V, ed Arboviruses of the California Complex and the Bunyamwera Group Bratislava, Slovakia: Publishing house of the Slovak Academy of Sciences, 1966**:333-335.

17. Beard JR, Trent M, Sam GA, Delpech VC. Self-reported morbidity of Barmah Forest virus infection on the north coast of New South Wales. **Med J Aust 1997**;167:525-8.

18. Becker FE. Tick-borne infections in Colorado. II. Survey of the occurrence of infections transmitted by the wood tick. **Colorado Med 1930**;27:87-95.

19. Bessaud M, Peyrefitte CN, Pastorino BA, Gravier P, Tock F, Boete F, Tolou HJ, Grandadam M. O'nyong-nyong Virus, Chad. **Emerg Infect Dis 2006**;12:1248-50.

20. Bhatt PN, Rodrigues FM. Chandipura: a new arbovirus isolated in India from patients with febrile illness. **Indian J Med Res 1967**;55:1295-305.

21. Bick MJ, Carroll JW, Gao G, Goff SP, Rice CM, MacDonald MR. Expression of the zinc-finger antiviral protein inhibits alphavirus replication. **J Virol 2003**;77:11555-62.

22. Boctor FN, Calisher CH, Peter JB. Dot-ELISA for serodiagnosis of human infections due to Western equine encephalitis virus. **J Virol Methods 1989**;26:305-11.

23. Boughton CR, Hawkes RA, Naim HM. Illness caused by a Barmah Forest-like virus in New South Wales. **Med J Aust 1988**;148:146-7.

24. Brighton SW, Simson IW. A destructive arthropathy following Chikungunya virus arthritis--a possible association. **Clin Rheumatol 1984**;3:253-8.

25. Briolant S, Garin D, Scaramozzino N, Jouan A, Crance JM. In vitro inhibition of Chikungunya and Semliki Forest viruses replication by antiviral compounds: synergistic effect of interferon-alpha and ribavirin combination. **Antiviral Res 2004**;61:111-7.

26. Broliden K, Tolfvenstam T, Norbeck O. Clinical aspects of parvovirus B19 infection. **J Intern Med 2006**;260:285-304.

27. Brummer-Korvenkontio M, Korhonen P, Hämeen-Anttila R. Ecology and phenology of mosquitoes (*Dipt. Culicidae*) inhabiting small pools in Finland. **1971**;28:51-73.

28. Brummer-Korvenkontio M, Kuusisto P. Onko Suomen Länsiosa säästynyt 'Pogostalta' ? [Has Western Finland been spared the 'Pogosta'?]. **Suom Laakaril 1981**;32:2606-7.

29. Brummer-Korvenkontio M, Saikku P. Mosquito-borne viruses in Finland. **Med Biol 1975**;53:279-81.

30. Brummer-Korvenkontio M, Saikku P, Korhonen P, Ulmanen I, Reunala T, Karvonen J. Arboviruses in Finland. IV. Isolation and characterization of Inkoo virus, a Finnish representative of the California group. **Am J Trop Med Hyg 1973**;22:404-13.

31. Brummer-Korvenkontio M, Vapalahti O, Kuusisto P, Saikku P, Manni T, Koskela P, Nygren T, Brummer-Korvenkontio H, Vaheri A. Epidemiology of Sindbis virus infections in Finland 1981-96: possible factors explaining a peculiar disease pattern. **Epidemiol Infect 2002**;129:335-45.

32. Buckley A, Dawson A, Moss SR, Hinsley SA, Bellamy PE, Gould EA. Serological evidence of West Nile virus, Usutu virus and Sindbis virus infection of birds in the UK. **J Gen Virol 2003**;84:2807-17.

33. Burke DS, Ramsburg HH, Edelman R. Persistence in humans of antibody to subtypes of Venezuelan equine encephalomyelitis (VEE) virus after immunization with attenuated (TC-83) VEE virus vaccine. **J Infect Dis 1977**;136:354-9.

34. Buskila D. Hepatitis C-associated arthritis. **Curr Opin Rheumatol 2000**;12:295-9.

35. Calisher CH. Medically important arboviruses of the United States and Canada. **Clin Microbiol Rev 1994**;7:89-116.

36. Calisher CH, Berardi VP, Muth DJ, Buff EE. Specificity of immunoglobulin M and G antibody responses in humans infected with eastern and western equine encephalitis viruses: application to rapid serodiagnosis. **J Clin Microbiol 1986**;23:369-72.

37. Calisher CH, el-Kafrawi AO, Al-Deen Mahmud MI, Travassos da Rosa AP, Bartz CR, Brummer-Korvenkontio M, Haksohusodo S, Suharyono W. Complex-specific immunoglobulin M antibody patterns in humans infected with alphaviruses. **J Clin Microbiol 1986**;23:155-9.

38. Calisher CH, Goodpasture HC. Human infection with Bhanja virus. **Am J Trop Med Hyg 1975**;24:1040-2.

39. Calisher CH, Karabatsos N, Lazuick JS, Monath TP, Wolff KL. Reevaluation of the western equine encephalitis antigenic complex of alphaviruses (family Togaviridae) as determined by neutralization tests. **Am J Trop Med Hyg 1988**;38:447-52.

40. Calisher CH, Meurman O, Brummer-Korvenkontio M, Halonen PE, Muth DJ. Sensitive enzyme immunoassay for detecting immunoglobulin M antibodies to Sindbis virus and further evidence that Pogosta disease is caused by a western equine encephalitis complex virus. **J Clin Microbiol 1985**;22:566-71.

41. Calisher CH, Shope RE, Brandt W, Casals J, Karabatsos N, Murphy FA, Tesh RB, Wiebe ME. Proposed antigenic classification of registered arboviruses I. Togaviridae, Alphavirus. **Intervirology 1980**;14:229-32.

42. Carrara AS, Gonzales G, Ferro C, Tamayo M, Aronson J, Paessler S, Anishchenko M, Boshell J, Weaver SC. Venezuelan equine encephalitis virus infection of spiny rats. **Emerg Infect Dis 2005**;11:663-9.

43. Causey OR, Maroja OM. Mayaro virus: a new human disease agent. III. Investigation of an epidemic of acute febrile illness on the river Guama in Para, Brazil, and isolation of Mayaro virus as causative agent. **Am J Trop Med Hyg 1957**;6:1017-23.

44. Charrel RN, de Lamballerie X, Raoult D. Chikungunya outbreaks--the globalization of vectorborne diseases. **N Engl J Med 2007**;356:769-71.

45. Chastel C. Erve and Eyach: two viruses isolated in France, neuropathogenic for man and widely distributed in Western Europe. **Bull Acad Natl Med 1998**;182:801,9; discussion 809-10.

46. Clarke DH, Casals J. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. **Am J Trop Med Hyg 1958**;7:561-73.

47. Coates DM, Makh SR, Jones N, Lloyd G. Assessment of assays for the serodiagnosis of Venezuelan equine encephalitis. **J Infect 1992**;25:279-89.

48. Cohen R, O'Connor RE, Townsend TE, Webb PA, McKey RW. Western equine encephalomyelitis; clinical observations in infants and children. **J Pediatr 1953**;43:26-34.

49. Cole FE,Jr. Inactivated eastern equine encephalomyelitis vaccine propagated in rolling-bottle cultures of chick embryo cells. **Appl Microbiol 1971**;22:842-5.

50. Condon RJ, Rouse IL. Acute symptoms and sequelae of Ross River virus infection in South-Western Australia: a follow-up study. **Clin Diagn Virol 1995**;3:273-84.

51. Contamin C, Brion JP, Bayle F, Morand P, Peo'ch M, Vialtel P. A case of arthritis caused by cytomegalovirus after kidney transplantation. **Transpl Infect Dis 2004**;6:87-9.

52. Copps SC, Giddings LE. Transplacental transmission of western equine encephalitis; report of a case. **Pediatrics 1959**;24:31-3.

53. Costenbader KH, Karlson EW. Epstein-Barr virus and rheumatoid arthritis: is there a link? **Arthritis Res Ther 2006**;8:204.

54. Cramp, S. *et al.* (eds.). **The Birds of the Western Palearctic**, I-IX ed., Oxford: Oxford University Press 1977-1994

55. Csepregi A, Nemesanszky E, Rojkovich B, Poor G. Rheumatoid arthritis and hepatitis B virus: evaluating the pathogenic link. **J Rheumatol 2001**;28:474-7.

56. Cuellar ML, Espinoza LR. Rheumatic manifestations of HIV-AIDS. **Baillieres Best Pract Res Clin Rheumatol 2000**;14:579-93.

57. Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or Rift Valley fever: an undescribed virus disease of sheep cattle and man from East Africa. **J Pathol Bacteriol 1931**;34:545-79.

58. Davis NL, Grieder FB, Smith JF, Greenwald GF, Valenski ML, Sellon DC, Charles PC, Johnston RE. A molecular genetic approach to the study of Venezuelan equine encephalitis virus pathogenesis. **Arch Virol Suppl 1994**;9:99-109.

59. de la Monte S, Castro F, Bonilla NJ, Gaskin de Urdaneta A, Hutchins GM. The systemic pathology of Venezuelan equine encephalitis virus infection in humans. **Am J Trop Med Hyg 1985**;34:194-202.

60. De Mucha-Macias J, Sanchez-Spindola I. Two human cases of laboratory infection with Mucambo virus. **Am J Trop Med Hyg 1965**;14:475-8.

61. Deller JJ,Jr, Russell PK. Chikungunya disease. **Am J Trop Med Hyg 1968**;17:107-11.

62. Deresiewicz RL, Thaler SJ, Hsu L, Zamani AA. Clinical and neuroradiographic manifestations of eastern equine encephalitis. **N Engl J Med 1997**;336:1867-74.

63. DeTulleo L, Kirchhausen T. The clathrin endocytic pathway in viral infection. **EMBO J 1998**;17:4585-93.

64. Dietz WH,Jr, Peralta PH, Johnson KM. Ten clinical cases of human infection with venezuelan equine encephalomyelitis virus, subtype I-D. **Am J Trop Med Hyg 1979**;28:329-34.

65. Doherty RL, Carley JG, Best JC. Isolation of Ross River virus from man. **Med J Aust 1972**;1:1083-4.

66. Doherty RL, Carley JG, Gorman BM. Studies of Arthropod-Borne Virus Infections in Queensland. Iv. further Serological Investigations of Antibodies to Group B Arboviruses in Man and Animals. **Aust J Exp Biol Med Sci 1964**;42:149-64.

67. Doherty RL, Gorman BM, Whitehead RH, Carley JG. Studies of Epidemic Polyarthritis: the Significance of Three Group a Arboviruses Isolated from Mosquitoes in Queensland. **Australas Ann Med 1964**;13:322-7.

68. Donaghy M. Neurologists and the threat of bioterrorism. **J Neurol Sci 2006**;249:55-62.

69. Dougados M, van der Linden S, Juhlin R, Huitfeldt B, Amor B, Calin A, Cats A, Dijkmans B, Olivieri I, Pasero G. The European Spondylarthropathy Study Group preliminary criteria for the classification of spondylarthropathy. **Arthritis Rheum 1991**;34:1218-27.

70. Edelman R, Ascher MS, Oster CN, Ramsburg HH, Cole FE, Eddy GA. Evaluation in humans of a new, inactivated vaccine for Venezuelan equine encephalitis virus (C-84). **J Infect Dis 1979**;140:708-15.

71. Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA. Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. **Am J Trop Med Hyg 2000**;62:681-5.

72. Ehrenkranz NJ, Ventura AK. Venezuelan equine encephalitis virus infection in man. **Annu Rev Med 1974**;25:9-14.

73. Ehrnst A, Peters CJ, Niklasson B, Svedmyr A, Holmgren B. Neurovirulent Toscana virus (a sandfly fever virus) in Swedish man after visit to Portugal. **Lancet 1985**;1:1212-3.

74. Englund JA, Breningstall GN, Heck LJ, Lazuick JS, Karabatsos N, Calisher CH, Tsai TF. Diagnosis of western equine encephalitis in an infant by brain biopsy. **Pediatr Infect Dis 1986**;5:382-4.

75. Enserink M. Infectious diseases. Massive outbreak draws fresh attention to little-known virus. **Science 2006**;311:1085.

76. Espmark A, Niklasson B. Ockelbo disease in Sweden: epidemiological, clinical, and virological data from the 1982 outbreak. **Am J Trop Med Hyg 1984**;33:1203-11.

77. Fauvel M, Artsob H, Calisher CH, Davignon L, Chagnon A, Skvorc-Ranko R, Belloncik S. California group virus encephalitis in three children from Quebec: clinical and serologic findings. **Can Med Assoc J 1980**;122:60-4.

78. Fazakerley JK. Pathogenesis of Semliki Forest virus encephalitis. **J Neurovirol 2002**;8 Suppl 2:66-74.

79. Feemster RF. Outbreak of encephalitis in man due to the Eastern virus of equine encephalomyelitis. **Am J Public Health 1938**;28:1403-10.

80. Feemster RF, Haymaker W. Eastern equine encephalitis. **Neurology 1958**;8:882-3.

81. Feeney KT, Murray KJ, Whittle AJ, Dowse GK. Reactive arthritis and vasculitis in a child due to Ross River virus infection. **Med J Aust 2004**;181:710.

82. Ferro C, Boshell J, Moncayo AC, Gonzalez M, Ahumada ML, Kang W, Weaver SC. Natural enzootic vectors of Venezuelan equine encephalitis virus, Magdalena Valley, Colombia. **Emerg Infect Dis 2003**;9:49-54.

83. Flexman JP, Smith DW, Mackenzie JS, Fraser JR, Bass SP, Hueston L, Lindsay MD, Cunningham AL. A comparison of the diseases caused by Ross River virus and Barmah Forest virus. **Med J Aust 1998**;169:159-63.

84. Flint SJ, Enquist LW, Racaniello VR, Skalka AM. **Principles of Virology**, 2nd ed., Washington: ASM Press 2004

85. Fothergill LD, Dingle JH, Farber S, Connerley ML. Human encephalitis caused by the virus of the Eastern variety of equine encephalomyelitis. **N Engl J Med 1938**;219:411-.

86. Fourie ED, Morrison JG. Rheumatoid arthritic syndrome after chikungunya fever. **S Afr Med J 1979**;56:130-2.

87. Frances SP, Cooper RD, Rowcliffe KL, Chen N, Cheng Q. Occurrence of Ross River virus and Barmah Forest virus in mosquitoes at Shoalwater Bay military training area, Queensland, Australia. **J Med Entomol 2004**;41:115-20.

88. Francy DB, Jaenson TG, Lundström JO, Schildt EB, Espmark A, Henriksson B, Niklasson B. Ecologic studies of mosquitoes and birds as hosts of Ockelbo virus in Sweden and isolation of Inkoo and Batai viruses from mosquitoes. **Am J Trop Med Hyg 1989**;41:355-63.

89. Fraser JR, Cunningham AL. Incubation time of epidemic polyarthritis. **Med J Aust 1980**;1:550-1.

90. Fraser JR, Cunningham AL, Mathews JD, Riglar A. Immune complexes and Ross River virus disease (epidemic polyarthritis). **Rheumatol Int 1988**;8:113-7.

91. Fraser JR, Cunningham AL, Muller HK, Sinclair RA, Standish HG. Glomerulonephritis in the acute phase of Ross River virus disease (epidemic polyarthritis). **Clin Nephrol 1988**;29:149-52.

92. Fraser JR, Tait B, Aaskov JG, Cunningham AL. Possible genetic determinants in epidemic polyarthritis caused by Ross River virus infection. **Aust N Z J Med 1980**;10:597-603.

93. Fulton JS, Burton AN. After effects of western equine encephalomyelitis infection in man. **Can Med Assoc J 1953**;69:268-72.

94. Garmashova N, Gorchakov R, Volkova E, Paessler S, Frolova E, Frolov I. The Old World and New World alphaviruses use different virus-specific proteins for induction of the transcriptional shutoff. **J Virol 2006**

95. Garoff H, Huylebroeck D, Robinson A, Tillman U, Liljestrom P. The signal sequence of the p62 protein of Semliki Forest virus is involved in initiation but not in completing chain translocation. **J Cell Biol 1990**;111:867-76.

96. Gear JH, Thomson PD, Hopp M, Andronikou S, Cohn RJ, Ledger J, Berkowitz FE. Congo-Crimean haemorrhagic fever in South Africa. Report of a fatal case in the Transvaal. **S Afr Med J 1982**;62:576-80.

97. Gerrard SR, Li L, Barrett AD, Nichol ST. Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. **J Virol 2004**;78:8922-6.

98. Getting VA. Equine encephalomyelitis in Massachusetts. **N Engl J Med 1941**;224:999-1006.

99. Goldfield M, Sussman O. The 1959 outbreak of Eastern encephalitis in New Jersey. I. Introduction and description of outbreak. **Am J Epidemiol 1968**;87:1-10.

100. Goldfield M, Welsh JN, Taylor BF. The 1959 outbreak of Eastern encephalitis in New Jersey. 5. The inapparent infection:disease ratio. **Am J Epidemiol 1968**;87:32-3.

101. Gordon SC, Lauter CB. Mumps arthritis: a review of the literature. **Rev Infect Dis 1984**;6:338-44.

102. Grimstad PR, Shabino CL, Calisher CH, Waldman RJ. A case of encephalitis in a human associated with a serologic rise to Jamestown Canyon virus. **Am J Trop Med Hyg 1982**;31:1238-44.

103. Guard RW, McAuliffe MJ, Stallman ND, Bramston BA. Haemorrhagic manifestations with Sindbis infection. Case report. **Pathology 1982**;14:89-90.

104. Gupta RK, Best J, MacMahon E. Mumps and the UK epidemic 2005. **BMJ 2005**;330:1132-5.

105. Hahn CS, Lustig S, Strauss EG, Strauss JH. Western equine encephalitis virus is a recombinant virus. **Proc Natl Acad Sci U S A 1988**;85:5997-6001.

106. Hammon WM, Reeves WC. California encephalitis virus, a newly described agent. I. Evidence of infection in man and other animals. **California Medicine 1952**;77:303-9.

107. Hammon WM, Reeves WC, Brookman B, Izumi EM, Gjullin CM. Isolation of the viruses of western equine and St. Louis encephalitis from *Culex tarsalis* mosquitoes. **Science 1941**;94:328-30.

108. Hanson RP. An epizootic of equine encephalomyelitis that occurred in Massachusetts in 1831. **Am J Trop Med Hyg 1957**;6:858-62.

109. Hardy JL. The ecology of western equine encephalomyelitis virus in the Central Valley of California, 1945-1985. **Am J Trop Med Hyg 1987**;37:18S-32S.

110. Harley D, Bossingham D, Purdie DM, Pandeya N, Sleigh AC. Ross River virus disease in tropical Queensland: evolution of rheumatic manifestations in an inception cohort followed for six months. **Med J Aust 2002**;177:352-5.

111. Harley D, Sleigh A, Ritchie S. Ross River virus transmission, infection, and disease: a cross-disciplinary review. **Clin Microbiol Rev 2001**;14:909,32, table of contents.

112. Hart KL, Keen D, Belle EA. An outbreak of Eastern Equine Encephalomyelitis in Jamaica, West Indies. I. Description Of human cases. **Am J Trop Med Hyg 1964**;13:331-4.

113. Hayashi M. Ubertragung des virus von encephalitis epidemica auf Affen [Infection of a monkey with an epidemic encephalitic virus]. **Proc Imp Acad Tokyo 1934**;10:41-4.

114. Heise MT, Simpson DA, Johnston RE. Sindbis-group alphavirus replication in periosteum and endosteum of long bones in adult mice. **J Virol 2000**;74:9294-9.

115. Hertig M, Sabin AB. Sandfly fever (pappataci, phlebotomus, three day fever). **In: Hoff EC, ed Preventive medicine in World War II Vol 7 Communicable Diseases Washington DC, Office of the Surgeon General, US Department of Army 1964**;9:109-74.

116. Hoch AL, Peterson NE, LeDuc JW, Pinheiro FP. An outbreak of Mayaro virus disease in Belterra, Brazil. III. Entomological and ecological studies. **Am J Trop Med Hyg 1981**;30:689-98.

117. Holden P, Hess AD. Cache Valley virus, a previously undescribed mosquito-borne agent. **Science 1959**;130:1187-8.

118. Hörling J, Vene S, Franzen C, Niklasson B. Detection of Ockelbo virus RNA in skin biopsies by polymerase chain reaction. **J Clin Microbiol 1993**;31:2004-9.

119. Howitt BF. Recovery of the virus of equine encephalomyelitis from the brain of a child. **Science 1938**;88:455-.

120. Johnson AJ, Martin DA, Karabatsos N, Roehrig JT. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody-based capture enzyme-linked immunosorbent assay. **J Clin Microbiol 2000**;38:1827-31.

121. Johnson RT, McFarland HF, Levy SE. Age-dependent resistance to viral encephalitis: studies of infections due to Sindbis virus in mice. **J Infect Dis 1972**;125:257-62.

122. Johnston RE, Peters CJ. Alphaviruses. In: Fields BN, Knipe DM, Howley PM. **Fields Virology**, 3rd ed., Philadelphia: Lippincott-Raven 1996

123. Jordan RA, Wagner JA, McCrumb FR. Eastern Equine Encephalitis: Report of a case with autopsy. **Am J Trop Med Hyg 1965**;14:470-4.

124. Journeaux SF, Brown WG, Aaskov JG. Prolonged infection of human synovial cells with Ross River virus. **J Gen Virol 1987**;68:3165-9.

125. Julkunen I, Brummer-Korvenkontio M, Hautanen A, Kuusisto P, Lindström P, Wager O, Penttinen K. Elevated serum immune complex levels in Pogosta disease, an acute alphavirus infection with rash and arthritis. **J Clin Lab Immunol 1986**;21:77-82.

126. Jupp PG, McIntosh BM. Quantitative experiments on the vector capability of Culex (Culex) pipiens fatigans Wiedemann with West Nile and Sindbis viruses. **J Med Entomol 1970**;7:353-6.

127. Katz IA, Hale GE, Hudson BJ, Ibels LS, Eckstein RP, Dermott PL. Glomerulonephritis secondary to Barmah Forest virus infection. **Med J Aust 1997**;167:21-3.

128. Kennedy AC, Fleming J, Solomon L. Chikungunya viral arthropathy: a clinical description. **J Rheumatol 1980**;7:231-6.

129. Kharitonova NN, Leonov YA. Omsk hemorrhagic fever (in Russian). **Amerind Publishing, New Delhi 1985**

130. Kistner O, Barrett N, Bruhmann A, Reiter M, Mundt W, Savidis-Dacho H, Schober-Bendixen S, Dorner F, Aaskov J. The preclinical testing of a formaldehyde inactivated Ross River virus vaccine designed for use in humans. **Vaccine 2007**;25:4845-52.

131. Kiwanuka N, Sanders EJ, Rwaguma EB, Kawamata J, Ssengooba FP, Najjemba R, Were WA, Lamunu M, Bagambisa G, Burkot TR, Dunster L, Lutwama JJ, Martin DA, Cropp CB, Karabatsos N, Lanciotti RS, Tsai TF, Campbell GL. O'nyong-nyong fever in south-central Uganda, 1996-1997: clinical features and validation of a clinical case definition for surveillance purposes. **Clin Infect Dis 1999**;29:1243-50.

132. Koprowski H, Cox HR. Human laboratory infection with Venezuelan equine encephalomyelitis virus. **N Engl J Med 1947**;236:647-54.

133. Laine M, Luukkainen R, Jalava J, Ilonen J, Kuusisto P, Toivanen A. Prolonged arthritis associated with Sindbis-related (Pogosta) virus infection. **Rheumatology (Oxford) 2000**;39:1272-4.

134. Laine M, Vainionpaa R, Oksi J, Luukkainen R, Toivanen A. The prevalence of antibodies against Sindbis-related (Pogosta) virus in different parts of Finland. **Rheumatology (Oxford) 2003**;42:632-6.

135. Lalitha P, Rathinam S, Banushree K, Maheshkumar S, Vijayakumar R, Sathe P. Ocular Involvement Associated With an Epidemic Outbreak of Chikungunya Virus Infection. **Am J Ophthalmol 2007**

136. Lanciotti RS, Ludwig ML, Rwaguma EB, Lutwama JJ, Kram TM, Karabatsos N, Cropp BC, Miller BR. Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. **Virology 1998**;252:258-68.

137. Lavergne A, de Thoisy B, Lacoste V, Pascalis H, Pouliquen JF, Mercier V, Tolou H, Dussart P, Morvan J, Talarmin A, Kazanji M. Mayaro virus: complete nucleotide sequence and phylogenetic relationships with other alphaviruses. **Virus Res 2006**;117:283-90.

138. Lee EY, Song CH, Choi SO. Acute polyarthritis associated with hantavirus infection. **Nephrol Dial Transplant 1999**;14:2204-5.

139. Leech RW, Harris JC, Johnson RM. 1975 encephalitis epidemic in North Dakota and western Minnesota. An epidemiologic, clinical, and neuropathologic study. **Minn Med 1981**;64:545-8.

140. Leon CA. Sequelae of Venezuelan equine encephalitis in humans: a four year follow-up. **Int J Epidemiol 1975**;4:131-40.

141. Li Y, Wang L, Li S, Chen X, Shen Y, Zhang Z, He H, Xu W, Shu Y, Liang G, Fang R, Hao X. Seco-pregnane steroids target the subgenomic RNA of alphavirus-like RNA viruses. **Proc Natl Acad Sci U S A 2007**;104:8083-8.

142. Liao M, Kielian M. Functions of the stem region of the Semliki Forest virus fusion protein during virus fusion and assembly. **J Virol 2006**;80:11362-9.

143. Liljeström P, Garoff H. Internally located cleavable signal sequences direct the formation of Semliki Forest virus membrane proteins from a polyprotein precursor. **J Virol 1991**;65:147-54.

144. Lindén H, Helle E, Helle P, Wikman M. Wildlife triangle scheme in Finland: methods and aims for monitoring wildlife populations. **Finnish Game Res 1996**;49:4-11.

145. Lindén H, Rajala P. Fluctuations and long-term trends in the relative densities of tetraonid populations in Finland, 1964-77. **Finnish Game Res 1981**;39:13-34.

146. Lindsay MD, Johansen CA, Smith DW, Wallace MJ, Mackenzie JS. An outbreak of Barmah Forest virus disease in the south-west of Western Australia. **Med J Aust 1995**;162:291-4.

147. Linn ML, Aaskov JG, Suhrbier A. Antibody-dependent enhancement and persistence in macrophages of an arbovirus associated with arthritis. **J Gen Virol 1996**;77:407-11.

148. Linn ML, Eble JA, Lubken C, Slade RW, Heino J, Davies J, Suhrbier A. An arthritogenic alphavirus uses the alpha1beta1 integrin collagen receptor. **Virology 2005**;336:229-39.

149. Linn ML, Mateo L, Gardner J, Suhrbier A. Alphavirus-specific cytotoxic T lymphocytes recognize a cross-reactive epitope from the capsid protein and can eliminate virus from persistently infected macrophages. **J Virol 1998**;72:5146-53.

150. Liu C, Voth DW, Rodina P, Shauf LR, Gonzalez G. A comparative study of the pathogenesis of western equine and eastern equine encephalomyelitis viral infections in mice by intracerebral and subcutaneous inoculations. **J Infect Dis 1970**;122:53-63.

151. Lopes OS, Coimbra TLM, Sacchetta LA, Calisher CH. Emergence of a new arbovirus disease in Brazil. **Am J Epidemiol 1978**;107:444-9.

152. Lundström JO. Mosquito-borne viruses in western Europe: a review. **J Vector Ecol 1999**;24:1-39.

153. Lundström JO, Lindström KM, Olsen B, Dufva R, Krakower DS. Prevalence of sindbis virus neutralizing antibodies among Swedish passerines indicates that thrushes are the main amplifying hosts. **J Med Entomol 2001**;38:289-97.

154. Lundström JO, Turell MJ, Niklasson B. Antibodies to Ockelbo virus in three orders of birds (Anseriformes, Galliformes and Passeriformes) in Sweden. **J Wildl Dis 1992**;28:144-7.

155. Lundström JO, Vene S, Espmark A, Engvall M, Niklasson B. Geographical and temporal distribution of Ockelbo disease in Sweden. **Epidemiol Infect 1991**;106:567-74.

156. Lundström K. Biology and application of alphaviruses in gene therapy. **Gene Ther 2005**;12 Suppl 1:S92-7.

157. Lury KM, Castillo M. Eastern equine encephalitis: CT and MRI findings in one case. **Emerg Radiol 2004**;11:46-8.

158. Luukkainen R, Laine M, Nirhamo J. Chronic arthritis after Sindbis-related (Pogosta) virus infection. **Scand J Rheumatol 2000**;29:399-400.

159. L'vov DK, Skvortsova TM, Berezina LK, Gromashevsky VL, Yakovlev BI, Gushchin BV, Aristova VA, Sidorova GA, Gushchina EL, Klimenko SM. Isolation of Karelian fever agent from Aedes communis mosquitoes. **Lancet 1984**;2:399-400.

160. L'vov DK, Skvortsova TM, Gromashevskii VL, Berezina LK, Iakovlev VI. Isolation of the causative agent of Karelian fever from Aedes sp. mosquitoes. **Vopr Virusol 1985**;30:311-3.

161. Lyer CGS, Rao RL, Work TH, Murthy DPN. Kyasanur Forest disease, VI: Pathological findings in three fatal human cases of Kyasanur Forest disease. **Indian J Med Sci 1959**;13:1011-22.

162. MacDonald GH, Johnston RE. Role of dendritic cell targeting in Venezuelan equine encephalitis virus pathogenesis. **J Virol 2000**;74:914-22.

163. Mackenzie JS, Lindsay MD, Coelen RJ, Broom AK, Hall RA, Smith DW. Arboviruses causing human disease in the Australasian zoogeographic region. **Arch Virol 1994**;136:447-67.

164. Malherbe H, Strickland-Cholmley M, Jackson AL. Sindbis virus infection in man. Report of a case with recovery of virus from skin lesions. **S Afr Med J 1963**;37:547-52.

165. Marshall ID, Woodroofe GM, Hirsch S. Viruses recovered from mosquitoes and wildlife serum collected in the Murray Valley of South-eastern Australia, February 1974, during an epidemic of encephalitis. **Aust J Exp Biol Med Sci 1982**;60:457-70.

166. Mathiot CC, Grimaud G, Garry P, Bouquety JC, Mada A, Daguisy AM, Georges AJ. An outbreak of human Semliki Forest virus infections in Central African Republic. **Am J Trop Med Hyg 1990**;42:386-93.

167. McIntosh BM, Jupp PG, Dickinson DB, McGillivray GM, Sweetnam J. Ecological studies on Sindbis and West Nile viruses in South Africa. I. Viral activity as revealed by infection of mosquitoes and sentinel fowls. **S Afr J Med Sci 1967**;32:1-14.

168. McIntosh BM, McGillivray GM, Dickinson DB, Malherbe H. Illness Caused by Sindbis and West Nile Viruses in South Africa. **S Afr Med J 1964**;38:291-4.

169. McIntosh BM, McGillivray GM, Dickinson DB, Taljaard JJ. Ecological studies on Sindbis and West Nile viruses in South Africa. IV. Infection in a wild avian population. **S Afr J Med Sci 1968**;33:105-12.

170. McLean DM, Donohue WL. Powassan virus: isolation of virus from a fatal case of encephalitis. **Can Med Assoc J 1959**;80:708-11.

171. Mirabel M, Vignaux O, Lebon P, Legmann P, Weber S, Meune C. Acute myocarditis due to Chikungunya virus assessed by contrast-enhanced MRI. **Int J Cardiol 2007**

172. Mitchell CJ, Niebylski ML, Smith GC, Karabatsos N, Martin D, Mutebi JP, Craig GB,Jr, Mahler MJ. Isolation of eastern equine encephalitis virus from Aedes albopictus in Florida. **Science 1992**;257:526-7.

173. Moore DL, Causey OR, Carey DE, Reddy S, Cooke AR, Akinkugbe FM, David-West TS, Kemp GE. Arthropod-borne viral infections of man in Nigeria, 1964-1970. **Ann Trop Med Parasitol 1975**;69:49-64.

174. Morrison TE, Fraser RJ, Smith PN, Mahalingam S, Heise MT. Complement contributes to inflammatory tissue destruction in a mouse model of Ross River virus-induced disease. **J Virol 2007**;81:5132-43.

175. Morrison TE, Whitmore AC, Shabman RS, Lidbury BA, Mahalingam S, Heise MT. Characterization of Ross River virus tropism and virus-induced inflammation in a mouse model of viral arthritis and myositis. **J Virol 2006**;80:737-49.

176. Muckenfuss RS, Armstrong C, McCordock HA. Encephalitis: Studies on experimental transmission. **Public Health Rep 1933**;48:1341-3.

177. Mulder DW, Parrott M, Thaler M. Sequelae of western equine encephalitis. **Neurology 1951**;1:318-27.

178. Mylonas AD, Brown AM, Carthew TL, McGrath B, Purdie DM, Pandeya N, Vecchio PC, Collins LG, Gardner ID, de Looze FJ, Reymond EJ, Suhrbier A. Natural history of Ross River virus-induced epidemic polyarthritis. **Med J Aust 2002**;177:356-60.

179. Ni H, Yun NE, Zacks MA, Weaver SC, Tesh RB, da Rosa AP, Powers AM, Frolov I, Paessler S. Recombinant alphaviruses are safe and useful serological diagnostic tools. **Am J Trop Med Hyg 2007**;76:774-81.

180. Niklasson B, Espmark A. Ockelbo disease: arthralgia 3-4 years after infection with a Sindbis virus related agent. **Lancet 1986**;1:1039-40.

181. Niklasson B, Espmark A, LeDuc JW, Gargan TP, Ennis WA, Tesh RB, Main AJ,Jr. Association of a Sindbis-like virus with Ockelbo disease in Sweden. **Am J Trop Med Hyg 1984**;33:1212-7.

182. Niklasson B, Espmark A, Lundström J. Occurrence of arthralgia and specific IgM antibodies three to four years after Ockelbo disease. **J Infect Dis 1988**;157:832-5.

183. Nimmannitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. **Am J Trop Med Hyg 1969**;18:954-71.

184. Nimmo JR. An unusual epidemic. **Med J Aust 1928**;1:549-50.

185. Nishioka K, Maruyama I, Sato K, Kitajima I, Nakajima Y, Osame M. Chronic inflammatory arthropathy associated with HTLV-I. **Lancet 1989**;1:441.

186. Nocton JJ, Miller LC, Tucker LB, Schaller JG. Human parvovirus B19-associated arthritis in children. **J Pediatr 1993**;122:186-90.

187. Norder H, Lundström JO, Kozuch O, Magnius LO. Genetic relatedness of Sindbis virus strains from Europe, Middle East, and Africa. **Virology 1996**;222:440-5.

188. O'Brien L. Inhibition of multiple strains of Venezuelan equine encephalitis virus by a pool of four short interfering RNAs. **Antiviral Res 2007**;75:20-9.

189. Ogen-Odoi A, Miller BR, Happ CM, Maupin GO, Burkot TR. Isolation of thogoto virus (Orthomyxoviridae) from the banded mongoose, Mongos mungo (Herpestidae), in Uganda. **Am J Trop Med Hyg 1999**;60:439-40.

190. Oker-Blom N, Brummer-Korvenkontio M. Inkoo virus. **In: Karabatsos N International catalogue of arboviruses (including certain other viruses of vertebrates) Texas: American Society of Tropical Medicine and Hygiene 1985**481-2.

191. Olson JG, Ksiazek TG, Suhandiman, Triwibowo. Zika virus, a cause of fever in Central Java, Indonesia. **Trans R Soc Trop Med Hyg 1981**;75:389-93.

192. Ozden S, Huerre M, Riviere JP, Coffey LL, Afonso PV, Mouly V, de Monredon J, Roger JC, El Amrani M, Yvin JL, Jaffar MC, Frenkiel MP, Sourisseau M, Schwartz O, Butler-Browne G, Despres P, Gessain A, Ceccaldi PE. Human muscle satellite cells as targets of chikungunya virus infection. **PLoS ONE 2007**;2:e527.

193. Paessler S, Aguilar P, Anishchenko M, Wang HQ, Aronson J, Campbell G, Cararra AS, Weaver SC. The hamster as an animal model for eastern equine encephalitis--and its use in studies of virus entrance into the brain. **J Infect Dis 2004**;189:2072-6.

194. Patel S, McLauchlin J, Casemore DP. A simple SDS-PAGE immunoblotting technique using an enhanced chemiluminescence detection system to identify polyclonal antibody responses to complex cryptosporidial antigen preparations following a monoclonal antibody retest and image overlay technique. **J Immunol Methods 1997**;205:157-61.

195. Phillips DA, Aaskov JG, Atkin C, Wiemers MA. Isolation of Kunjin virus from a patient with a naturally acquired infection. **Med J Aust 1992**;157:190-1.

196. Phillips DA, Murray JR, Aaskov JG, Wiemers MA. Clinical and subclinical Barmah Forest virus infection in Queensland. **Med J Aust 1990**;152:463-6.

197. Pialoux G, Gauzere BA, Jaureguiberry S, Strobel M. Chikungunya, an epidemic arbovirosis. **Lancet Infect Dis 2007**;7:319-27.

198. Piliero PJ, Brody J, Zamani A, Deresiewicz RL. Eastern equine encephalitis presenting as focal neuroradiographic abnormalities: case report and review. **Clin Infect Dis 1994**;18:985-8.

199. Pinheiro FP, Freitas RB, Travassos da Rosa JF, Gabbay YB, Mello WA, LeDuc JW. An outbreak of Mayaro virus disease in Belterra, Brazil. I. Clinical and virological findings. **Am J Trop Med Hyg 1981**;30:674-81.

200. Platteborze PL, Kondig JP, Schoepp RJ, Wasieloski LP. Comparative sequence analysis of the eastern equine encephalitis virus pathogenic strains FL91-4679 and GA97 to other North American strains. **DNA Seq 2005**;16:308-20.

201. Putkuri N, Brummer-Korvenkontio M, Vapalahti O. [Inkoo virus: A mosquito-borne cause of CNS infection in Finland]. **Suom Laakaril 2004**;21-22:2247-50.

202. Rao V, Hinz ME, Roberts BA, Fine D. Toxicity assessment of Venezuelan Equine Encephalitis virus vaccine candidate strain V3526. **Vaccine 2006**;24:1710-5.

203. Reid DM, Reid TM, Brown T, Rennie JA, Eastmond CJ. Human parvovirus-associated arthritis: a clinical and laboratory description. **Lancet 1985**;1:422-5.

204. Reiter P, Fontenille D, Paupy C. Aedes albopictus as an epidemic vector of chikungunya virus: another emerging problem? **Lancet Infect Dis 2006**;6:463-4.

205. Rice CM, Strauss JH. Nucleotide sequence of the 26S mRNA of Sindbis virus and deduced sequence of the encoded virus structural proteins. **Proc Natl Acad Sci U S A 1981**;78:2062-6.

206. Rivers TM, Schwentker FF. Louping ill in man. **J Exp Med 1934**;59:669-78.

207. Roehrig JT. Development of an enzyme-linked immunosorbent assay for the identification of arthropod-borne togavirus antibodies. **J Gen Virol 1982**;63:237-40.

208. Roehrig JT, Gorski D, Schlesinger MJ. Properties of monoclonal antibodies directed against the glycoproteins of Sindbis virus. **J Gen Virol 1982**;59:421-5.

209. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. **J Hyg (Lond) 1956**;54:177-91.

210. Rwaguma EB, Lutwama JJ, Sempala SD, Kiwanuka N, Kamugisha J, Okware S, Bagambisa G, Lanciotti R, Roehrig JT, Gubler DJ. Emergence of epidemic O'nyong-nyong fever in southwestern Uganda, after an absence of 35 years. **Emerg Infect Dis 1997**;3:77.

211. Ryan PA, Kay BH. Vector competence of mosquitoes (Diptera: Culicidae) from Maroochy Shire, Australia, for Barmah Forest virus. **J Med Entomol 1999**;36:856-60.

212. Sabin AB. Research on dengue during World War II. **Am J Trop Med Hyg 1952**;1:30-50.

213. Sanmartin-Barberi C, Groot H, Osorno-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalomyelitis virus. **Am J Trop Med Hyg 1954**;3:283-93.

214. Sariola M, Saraste J, Kuismanen E. Communication of post-Golgi elements with early endocytic pathway: regulation of endoproteolytic cleavage of Semliki Forest virus p62 precursor. **J Cell Sci 1995**;108 (Pt 6):2465-75.

215. Schaeffer M, Gadjusek DC, Lema AB, Eichenwald H. Epidemic jungle fevers among Okinawan colonists in the Bolivian rain forest. I. Epidemiology. **Am J Trop Med Hyg 1959**;8:372-96.

216. Schultz DR, Barthal JS, Garrett G. Western equine encephalitis with rapid onset of parkinsonism. **Neurology 1977**;27:1095-6.

217. Scott TW, Weaver SC. Eastern equine encephalomyelitis virus: epidemiology and evolution of mosquito transmission. **Adv Virus Res 1989**;37:277-328.

218. Selden SM, Cameron AS. Changing epidemiology of Ross River virus disease in South Australia. **Med J Aust 1996**;165:313-7.

219. Seyhan AA, Vitiello D, Shields MT, Burke JM. Ribozyme inhibition of alphavirus replication. **J Biol Chem 2002**;277:25957-62.

220. Shinefield HR, Townsend TE. Transplacental transmission of western equine encephalomyelitis. **J Pediatr 1953**;43:21-5.

221. Shore H. O'nyong-nyong fever: an epidemic virus disease in East Africa. III. Some clinical and epidemiological observations in the northern province of Uganda. **Trans R Soc Trop Med Hyg 1961**;55:361-73.

222. Smithburn KC, Hughes TP, Burke AW, Paul JH. A neurotropic virus isolated from the blood of a native of Uganda. **Am J Trop Med 1940**;20:471-92.

223. Smithburn KC, Kokernot RH, Weinbren MP, De Meillon B. Studies on arthropod-borne viruses of Tongaland. IX. Isolation of Wesselsbron virus from a naturally infected human being and from Aedes (Banksinella) circumluteolus Theo. **S Afr J Med Sci 1957**;22:113-20.

224. Smithburn KC, Mahaffy AF, Paul JH. Bwamba fever and its causative virus. **Am J Trop Med 1941**;21:75-90.

225. Somekh E, Glode MP, Reiley TT, Tsai TF. Multiple intracranial calcifications after western equine encephalitis. **Pediatr Infect Dis J 1991**;10:408-9.

226. Stokes A, Bauer JH, Hudson JH. Transmission of yellow fever to *Macacus Rhesus*, preliminary note. **JAMA 1928**;90:253-4.

227. Strauss EG, Rice CM, Strauss JH. Complete nucleotide sequence of the genomic RNA of Sindbis virus. **Virology 1984**;133:92-110.

228. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication, and evolution. **Microbiol Rev 1994**;58:491-562.

229. Suhrbier A, La Linn M. Clinical and pathologic aspects of arthritis due to Ross River virus and other alphaviruses. **Curr Opin Rheumatol 2004**;16:374-9.

230. Sundaram MB, Siemens P. Lateralized EEG abnormalities in western equine encephalitis. **Can Med Assoc J 1984**;131:186, 188.

231. Taubitz W, Cramer JP, Kapaun A, Pfeffer M, Drosten C, Dobler G, Burchard GD, Loscher T. Chikungunya fever in travelers: clinical presentation and course. **Clin Infect Dis 2007**;45:e1-4.

232. Taylor RM, Hurlbut HS, Work TH, Kingston JR, Frothingham TE. Sindbis virus: a newly recognized arthropodtransmitted virus. **Am J Trop Med Hyg 1955**;4:844-62.

233. Taylor SF, Patel PR, Herold TJ. Recurrent arthralgias in a patient with previous Mayaro fever infection. **South Med J 2005**;98:484-5.

234. Tesh RB, Watts DM, Russell KL, Damodaran C, Calampa C, Cabezas C, Ramirez G, Vasquez B, Hayes CG, Rossi CA, Powers AM, Hice CL, Chandler LJ, Cropp BC, Karabatsos N, Roehrig JT, Gubler DJ. Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South America. **Clin Infect Dis 1999**;28:67-73.

235. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. **Nucleic Acids Res 1994**;22:4673-80.

236. Thompson WH, Kalfayan B, Anslow RO. Isolation of California encephalitis group virus from a fatal human illness. **Am J Epidemiol 1965**;81:245-53.

237. Tingle AJ, Allen M, Petty RE, Kettyls GD, Chantler JK. Rubella-associated arthritis. I. Comparative study of joint manifestations associated with natural rubella infection and RA 27/3 rubella immunisation. **Ann Rheum Dis 1986**;45:110-4.

238. Tsai T, Monath TP. Alphaviruses. In: Richman DD, Whitley RJ, Hayden FG. **Clinical virology**, 1st ed., New York: Churchill Livingstone 1987

239. Turunen M, Kuusisto P, Uggeldahl PE, Toivanen A. Pogosta disease: clinical observations during an outbreak in the province of North Karelia, Finland. **Br J Rheumatol 1998**;37:1177-80.

240. van den Bosch C, Lloyd G. Chikungunya fever as a risk factor for endemic Burkitt's lymphoma in Malawi. **Trans R Soc Trop Med Hyg 2000**;94:704-5.

241. van Riel PLCM. **EULAR Handbook of Clinical Assessments in Rheumatoid Arthritis**, 1st ed., The Netherlands: Van Zuiden Communications B.V. 2000

242. Vene S, Franzen C, Niklasson B. Development of specific antibody patterns and clinical symptoms following Ockelbo virus infection. **Arch Virol 1994**;134:61-71.

243. Vogel P, Kell WM, Fritz DL, Parker MD, Schoepp RJ. Early events in the pathogenesis of eastern equine encephalitis virus in mice. **Am J Pathol 2005**;166:159-71.

244. Wahlberg P, Carlsson SA, Granlund H, Jansson C, Linden M, Nyberg C, Nyman D. TBE in Aland Islands 1959-2005: Kumlinge disease. **Scand J Infect Dis 2006**;38:1057-62.

245. Wahlberg P, Salminen A, Weckström P, Oker-Blom N. Diphasic Tick-Borne Meningo-Encephalitis, Kumlinge Disease, in the Aland Islands. Diagnosis, Clinical Features, and Epidemiology. **Acta Med Scand 1964**;412 (Suppl):275-86.

246. Wang E, Paessler S, Aguilar PV, Carrara AS, Ni H, Greene IP, Weaver SC. Reverse transcription-PCR-enzyme-linked immunosorbent assay for rapid detection and differentiation of alphavirus infections. **J Clin Microbiol 2006**;44:4000-8.

247. Wang G, Hernandez R, Weninger K, Brown DT. Infection of cells by Sindbis virus at low temperature. **Virology 2007**;362:461-7.

248. Watson TM, Kay BH. Vector competence of Aedes notoscriptus (Diptera: Culicidae) for Barmah Forest virus and of this species and Aedes aegypti (Diptera: Culicidae) for dengue 1-4 viruses in Queensland, Australia. **J Med Entomol 1999**;36:508-14.

249. Weaver SC, Ferro C, Barrera R, Boshell J, Navarro JC. Venezuelan equine encephalitis. **Annu Rev Entomol 2004**;49:141-74.

250. Weaver SC, Rico-Hesse R, Scott TW. Genetic diversity and slow rates of evolution in New World alphaviruses. **Curr Top Microbiol Immunol 1992**;176:99-117.

251. Weaver SC, Salas R, Rico-Hesse R, Ludwig GV, Oberste MS, Boshell J, Tesh RB. Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America. VEE Study Group. **Lancet 1996**;348:436-40.

252. Wenger F. Venezuelan equine encephalitis. **Teratology 1977**;16:359-62.

253. Willems WR, Kaluza G, Boschek CB, Bauer H, Hager H, Schutz HJ, Feistner H. Semliki forest virus: cause of a fatal case of human encephalitis. **Science 1979**;203:1127-9.

254. Williams MC, Woodall JP, Corbet PS, Gillett JD. O'nyong-Nyong Fever: an Epidemic Virus Disease in East Africa. 8. Virus Isolations from Anopheles Mosquitoes. **Trans R Soc Trop Med Hyg 1965**;59:300-6.

255. Williams MC, Woodall JP, Gillett JD. O'nyong-Nyong Fever: an Epidemic Virus Diesease in East Africa. 7. Virus Isolations from Man and Serological Studies Up to July 1961. **Trans R Soc Trop Med Hyg 1965**;59:186-97.

256. Winter WD. Eastern equine encephalomyelitis in Massachusetts in 1955; report of two cases in infants. **N Engl J Med 1956**;255:262-7.

257. Woodall JP, Williams MC, Ellice JM. Sindbis Infection in Man. **East African Virus Research Institute Report 1962**;12:17-.

258. Woolf AD, Campion GV, Chishick A, Wise S, Cohen BJ, Klouda PT, Caul O, Dieppe PA. Clinical manifestations of human parvovirus B19 in adults. **Arch Intern Med 1989**;149:1153-6.

259. Xu P, Wang Y, Zuo J, Lin J, Xu P. New strains of Banna virus. Isolation from patients with fever and encephalitis in Yunnan province (in chinese). **Chin J Virol 1990**;6:27-33.

260. Zarate ML, Scherer WF. Contact-spread of Venezuelan equine encephalomyelitis virus among cotton rats via urine or feces and the naso- or oropharynx. A possible transmission cycle in nature. **Am J Trop Med Hyg 1968**;17:894-9.

261. Zeller HG, Diallo M, Angel G, Traore-Lamizana M, Thonnon J, Digoutte JP, Fontenille D. Ngari virus (Bunyaviridae: Bunyavirus). First isolation from humans in Senegal, new mosquito vectors, its epidemiology. **Bull Soc Pathol Exot 1996**;89:12-6.

262. Zhang Z, Burke JM. Inhibition of viral replication by ribozyme: mutational analysis of the site and mechanism of antiviral activity. **J Virol 2005**;79:3728-36.

263. Zhou G, Liang G, Li L. Complete nucleotide sequence of the nonstructural gene of alphavirus YN87448 strain isolated in China and its relationship to other Sindbis viruses. **Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 1999**;13:314-20.

264. Zilber LA, Soloviev VD. Far Eastern tick-borne spring-summer (spring) encephalitis. **American Review of Soviet Medicine Special 1946**1-80.

265. Zytoon EM, el-Belbasi HI, Matsumura T. Transovarial transmission of chikungunya virus by Aedes albopictus mosquitoes ingesting microfilariae of Dirofilaria immitis under laboratory conditions. **Microbiol Immunol 1993**;37:419-21.

ORIGINAL PUBLICATIONS

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