California encephalitis orthobunyaviruses in northern Europe

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It is better to understand a little
than to misunderstand a lot
-Anatole France

Table of Contents

List of original publications

This thesis is based on the following articles, which are referred in the text by Roman numerals (I-IV).

I. Putkuri, N., Vaheri, A. and Vapalahti, O. 2007. Prevalence and protein specificity of human antibodies to Inkoo virus infection. *Clinical and Vaccine Immunology*, 14(12), 1555-62.

II. Putkuri, N., Kurkela, S., Levanov, L., Huhtamo, E., Vaheri, A. Sironen, T. and Vapalahti, O. 2014. Isolation and characterization of a California encephalitis serogroup orthobunyavirus from Finnish mosquitoes. *Infection*, *Genetics and Evolution*. Mar; 22:164-73.

III. Putkuri, N., Kantele, A., Levanov, L., Brummer-Korvenkontio, M., Vaheri, A. and Vapalahti, O. 2016. Acute human Inkoo and Chatanga virus infections, Finland. *Emerging Infectious Disease*. May;22(5):810-7.

IV. Evander, M., Putkuri, N., Eliasson, M., Vapalahti, O. and Ahlm, C. 2016. Seroprevalence and risk factors for Inkoo virus in Northern Sweden. *American Journal of Tropical Medicine and Hygiene*. May 4;94(5):1103-6.

List of abbreviations

CNS	central nervous system
CPE	cytopathogenic effect
Cx.	*Culex*
EIA	enzyme immunoassay
ELISA	enzyme-linked immunosorbent assay
Gc protein	carboxy-terminal glycoprotein
Gn protein	amino-terminal glycoprotein
HI	hemagglutination inhibition test
ICTV	International Committee on Taxonomy of Viruses
IFA	indirect immunofluorescence assay
IgG	immunoglobulin G
IgM	immunoglobulin M
kb	kilobase
kDa	kilodalton
L protein	large segment coded RNA-dependent RNA polymerase
N protein	nucleocapsid protein
NSm	non-structural protein, M segment
NSs	non-structural protein, S segment
PRNT50	plaque reduction neutralization assay (50% reduction)
Och.	*Ochlerotatus*
RdRp	RNA-dependent RNA polymerase
RNAi	RNA interference
RNP	ribonucleoprotein

All the abbreviations for virus names are listed in APPENDIX 1 (Page 86).

Abstract

Inkoo virus is a mosquito-borne virus first isolated in Finland in 1964. It belongs to the California serogroup (genus *Orthobunyavirus*, family *Bunyaviridae*), which includes many important human pathogens and is described especially in the USA. The association of Inkoo virus infection with clinical disease has not been confirmed, but occasional cases of meningitis and encephalitis have been diagnosed. However, the true incidence of acute infections is not known because ongoing research and laboratory diagnostics of INKV infections have been neglected for decades in the countries where the virus circulates.

We established a serological test (IFA) to detect Inkoo virus antibodies, and we also expressed the Inkoo virus N, Gc and Gn proteins with a baculovirus expression system to study protein-specific antibody responses. In a seroprevalence study, antibody prevalence in humans in Finland and Sweden showed that 40-50% of the population had been infected with Inkoo virus or a related California serogroup virus. The seroprevalence was higher in older age groups, and in Finland, the prevalence increased northwards. We found that acute-phase sera had a distinct granular fluorescence pattern in IgG IFA, whereas those with pre-existing immunity showed a diffuse pattern. Using recombinant Inkoo virus proteins as antigens, we noticed that the antibody response was predominantly against the N protein in early infection and also towards the Gc protein in the later stages of infection.

We discovered a new California encephalitis virus isolate of the genus *Orthobunyavirus* in Finland from mosquitoes collected in 2007 and 2008 from Ilomantsi and Sotkamo municipalities in Eastern Finland. Four isolates were obtained in 2007: M07-1, M07-2-83, M07-2-84 and S07-1. One isolate, ILO-8-3, is from 2008 collections, and it exhibited minimal differences from previous Finnish strains in phylogenetic analysis. The vector for the virus is uncertain, but it is most likely *Ochlerotatus sp*. The new isolates were named the Möhkö isolates of Chatanga virus since the genetic and serological findings suggested that the new virus isolates were most closely related to clusters of Russian orthobunyavirus isolates named Chatanga virus (99% N protein identity) and then to Snowshoe hare virus and La Crosse virus (93% and 90% N protein identity, respectively).

We screened serum samples for INKV IgM antibodies, using samples from patients with febrile illness or neurological symptoms collected in the summertime between 2001 and 2013 to study the frequency of acute INKV infections and the clinical picture of the patients. We found the frequency to be under 1%, but interestingly, not only INKV but also CHATV were confirmed to cause human infections. Most patients were not hospitalized, and they had visited a doctor most often once, indicating very mild symptoms. The patients suffered from fever, headache, nausea/vomiting, disorientation/drowsiness, and seizures and had small changes in their EEG. INKV infections were more severe in children under 16 years, and CHATV infections were more severe in adults.

In this study, we found a new mosquito-borne virus (CHATV) from Finland and showed for the first time that this virus was associated with a clinical disease. In addition, we described the clinical picture of INKV infection and showed that the infection is more severe in children if neurologic symptoms appear. These viruses are common in Finland, and their association with clinical disease in the summertime should not be forgotten.

Review of the literature

1 Introduction

1.1 California serogroup

California encephalitis virus (CEV) group, known as the California serogroup, belongs to genus O*rthobunyavirus* in the family *Bunyaviridae*. The serogroup consists of 13 antigenically related but distinct virus isolates (Table 1). Phylogenetically, these viruses differ less than 10% in the amino acid sequences of their nucleocapsid proteins, and therefore, they all are classified as members of the California encephalitis virus species (as defined by ICTV). All virus isolates are transmitted by *Aedes* or *Ochlerotatus* mosquitoes, and many of them cause febrile illness or central nervous system (CNS) disease in humans (LeDuc 1987, 60S-68S)(LeDuc 1987). The majority of these viruses circulate in North America, but a few representatives are also found in Europe, Africa and Asia (Table 1). Most of the viruses were recovered decades ago, and despite their association with clinical illness, they have remained understudied and underdiagnosed in many countries.

1.2 Arthropods and infectious diseases

Many arthropods are associated with significant health problems for humans and animals, but only since the late 19th Century, when the so-called "golden age of medical entomology" started, have arthropods been shown to carry pathogens and demonstrated to spread diseases (Weaver et al. 2004). Although most often, mosquitoes, sandflies or ticks are involved in arthropod-borne diseases, a wide variety of arthropods can cause problems directly (blood feeding, biting, stinging) or indirectly (annoyance, crawling over, defecating upon and in stored food) for humans and animals.

Arthropod-borne diseases are predominantly caused by a diverse group of viruses and bacteria but also by protozoa (malaria, trypanosomiasis, babesiosis) and filarial nematodes (river blindness, dog heart worm). Contact with arthropods not only causes transmission of the pathogens but also causes other medical problems, i.e., allergic reactions, inflammations and secondary infections in the bite area (Mullen, Durden 2002). There are two routes of transmission of the infectious agent to the new host: mechanical or biological transmission. The latter is by far the most efficient and common way. In biological transmission, the pathogen infects and multiplies in an arthropod and is transmitted further by blood-feeding. Mechanical transmission spreads the pathogens via exodigestive secretion or with contaminated mouthparts (Mullen, Durden 2002, Reisen 2002).

Table 1. California serogroup isolates, their original isolation place and year, current geographical distribution area.

Isolate	Year and place of isolation	Original isolate	Distribution	Reference
California encephalitis virus (BFS-91)	1943 California, USA	*Ochlerotatus melanimon (dorsalis)*	California, Texas, Utah, Colorado, New Mexico, Alaska	(Hammon, Reeves & Sather 1952)
Trivittatus virus (Eklund strain)	1948 North Dakota, USA	*Ochlerotatus trivittatus*	USA	(Sudia et al. 1971, Karabatsos 1985)
Melao virus (TRVL9375)	195 Trinidad	*Ochlerotatus scapularis*	Trinidad, Brazil, Argentina	(Spence et al. 1962b)
Tahyna virus (92-Bardos)	1958 Czechoslovakia	*Ochlerotatus caspius*	Europe, Southern Siberia, Far East, China, Africa	(Bardos, Danielova 1959)
Snowshoe hare virus	1958 Montana, USA	*Lepus americanus*	North East of USA, Canada	(Burgdorfer, Newhouse & Thomas 1961)
San Angelo virus (20230)	1958 Texas, USA	*Anopheles pseudopunctipennis*	Texas, USA	(Sudia et al. 1971)
South river virus (NJO-94F)	1960 New Jersey, USA	*Anopheles crucians*	North East of USA	(Sudia et al. 1971)
La Crosse virus (LaX 177)	1960 Wisconsin, USA	Human brain tissues taken at autopsy	Upper Midwest and Eastern USA	(Thompson, Kalfayan & Anslow 1965)
Jamestown canyon virus (61V2235)	1961 Colorado, USA	*Culiseta inornata*	USA	(Karabatsos 1985)
Keystone virus (B64-5587.05)	1964 Florida, USA	*Ochlerotatus atlanticus tormentor*	South USA	(Bond et al. 1966)
Inkoo virus (KN3641)	1964 Finland	*Ochlerotatus Commis/punctor*	Finland, Sweden, Norway, Russia	(Brummer-Korvenkontio et al. 1973, Niklasson et al. 1985, Traavik, Mehl & Wiger 1978)
Serra do Navio virus (BeAr 103645)	1966 Amapa, Brazil	*Ochlerotatus fulvus*	Brazil	(LeDuc 1987, Karabatsos 1985)
Chatanga virus (LEIV-9843)	1983 Russia, Murmansk	*Aedes sp.*	Russia, Finland	(Lavrent'ev et al. 2008, Putkuri et al. 2014)

Viruses that are transmitted by arthropods are referred to as arthropod-borne viruses, or arboviruses. Arboviruses are categorized further by the arthropod they are carried by, i.e., mosquito-borne viruses or tick-borne viruses. The arthropod that carries the pathogen is referred to as the vector that can spread the pathogen, and hence these viruses cause vector-borne diseases (Reisen 2002).

Mosquitoes are found all over the world, and there are over 3500 species and 43 genera recognized. In phylum *Arthropoda*, mosquitoes belong to class *Insecta*, order *Diptera* and

family *Culicidae* (Zhang 2011). Significant vector species spreading infectious agents exist in the *Aedes, Ochlerotatus*, *Stegomyia, Anopheles*, *Culex, Haemagogus*, *Mansonia*, *Psorophora* and *Sobethes* genera (Service 2008).

Mosquitoes have four stages in their life cycles. Female mosquito lay eggs (I) after a blood-meal and can produce eggs several times during their lifetimes. Eggs are laid in the water or on damp surfaces, and it may take 2-14 days (*Anopheles* and *Culex*) or even months (*Aedes* and *Psorophora*) before the eggs hatch to larvae (II) depending on the temperature and flooding. The mosquitoes go through four larval forms before developing to pupae (III), and either larvae or pupae can survive desiccation. Finally, adult mosquitoes are developed 7-26 days from the egg hatching (Service 2008).

Maintenance of mosquito-borne viruses occurs via vertical and/or horizontal transmission mechanisms. Uninfected female mosquitoes are infected with viruses through blood-meals from infected animal hosts with sufficient viremia and are able to spread the virus further to new uninfected hosts (horizontal transmission). In addition, viruses are transmitted by transovarial transmission from infected female mosquitoes to their eggs, and the virus passes through all the mosquito life stages to adult mosquitoes (vertical transmission). This route produces both infected male and female mosquitoes. Male mosquitoes carrying the virus may infect female mosquitoes in mating (venereal transmission) (Reisen 2002, Borucki et al. 2002). Virus maintenance may involve several transmission routes in the natural life cycle.

Diseases caused by mosquito-borne viruses are a major global health concern. Dengue and Zika viruses (flaviviruses) and Chikungunya virus (alphavirus) are among the most common and widespread causative agents of mosquito-borne diseases. Sindbis virus (alphavirus), a causative agent of Pogosta disease, is the most common mosquito-borne virus in northern Europe.

2 Family Bunyaviridae

Family *Bunyaviridae* is a large group of viruses mainly transmitted by arthropods. The family includes over 350 viruses in 5 genera, named *Hantavirus, Nairovirus, Orthobunyavirus, Phlebovirus* and *Tospovirus* (King et al. 2012). *Hantavirus* is the only genus of the family carried by rodents and insectivores; the other genera are carried by different arthropods (mosquitoes, ticks, sandflies and thrips). Nairoviruses are predominantly transmitted by ticks; orthobunyaviruses are transmitted by mosquitoes; phleboviruses are transmitted by sandflies; and tospoviruses are transmitted by thrips. Although the principal vectors for each genus are those mentioned above, other arthropods have been recognized as vectors as well (Elliott and Plyusnin 2011).

All viruses in the family *Bunyaviridae* have a negative-sense, single-stranded, trisegmented RNA genome. The segments are called large (L), medium (M) and small (S). Terminal sequences of segments are conserved and genus-specific. In addition, the 3' and 5' terminal sequences are complementary to each other. The coding strategy is specific for structural proteins within the family. The L segment encodes RNA-dependent RNA polymerase (RdRp); the M segment encodes the polyprotein, which is cleaved into membrane glycoproteins (Gc and Gn); and the S segment encodes the nucleocapsid protein (N). The coding strategy varies depending on when/if the non-structural proteins NSs and NSm are encoded. While bunyaviruses share the same genomic structure, the sizes of the proteins and the length of the RNA segments differs between the genera but are similar within each genus (King et al. 2012, Schmaljohn, Hooper 2007).

Morphologically, bunyavirus particles are enveloped, spherical or pleomorphic and 80–120 nm in diameter (Murphy, Harrison & Whitfield 1973). Virions have two surface glycoproteins (Gc and Gn) and two internal proteins; the nucleocapsid and the L protein. Genomic segments are encapsidated with the N protein to form ribonucleoproteins (RNPs).

Bunyaviruses are causative agents of wide variety of human, animal and plant diseases. In humans, hantaviruses cause hantaviral cardiopulmonary syndrome (HCPS) and haemorrhagic fever with renal syndrome (HFRS) (Vaheri et al. 2013). Orthobunyaviruses and phleboviruses cause mainly subclinical infections or mild febrile illness, similar to many other arboviruses, but in some cases, encephalitis may occur (La Crosse virus, Oropouche virus, Toscana virus)(Balfour et al. 1973, Bernardes-Terzian et al. 2009, Ehrnst et al. 1985). However, more severe diseases, such as haemorrhagic fevers, have been described for the Nairovirus, Phlebovirus and Orthobunyavirus genera (Crimean-Congo haemorrhagic fever virus, Rift Valley fever virus, Ngari virus)(Whitehouse 2004, Chevalier et al. 2010, Gerrard et al. 2004).

The *Bunyaviridae* family also includes important veterinary pathogens that can cause abortion, foetal abnormalities and gastroenteritis in cattle and sheep (Akabane virus, Schmallenberg virus, Cache valley virus (orthobunyaviruses), Rift Valley virus (Phlebovirus), and Nairobi sheep disease(Nairovirus))(Parsonson, Della-Porta & Snowdon 1977, Chung et al. 1990, Bird et al. 2009, Marczinke, Nichol 2002). The plant pathogenic tospoviruses have been a cause of massive losses in the yield and quality of vegetable and legume crops in many parts of the world because of the expanding distribution of the vector, western flower thrips (Pappu, Jones & Jain 2009).

3 Genus Orthobunyavirus

3.1 Geographical distribution and reservoir hosts

Genus *Orthobunyavirus* is a large group of viruses containing more than 200 different virus isolates in 48 different virus species. All isolates are divided into 18 serogroups; Anopheles A and B, Bakau, Bwamba, Bunyamwera, California, Capim, Gamboa, Group C, Guama, Koongol, Patois, Olifantsvlei, Minatitlan, Nyando, Tete, Turlock and Simbu (King et al. 2012), as well as two possibly new serogroups, Wyeomyia and Mapputta (figure 1). All viruses within each serogroup are antigenically related but distinct. Orthobunyaviruses have been isolated all over the world from a large variety of vertebrates, leading to a large number of virus isolates (APPENDIX 1). Most viruses are transmitted by mosquitoes, but exceptions exist. Many viruses in the Simbu serogroup are transmitted by culicoides (biting midges), the Kaeng Khoi virus of the Nyando serogroup is transmitted by bed bugs, and several viruses of the Tete serogroup are transmitted by ticks. Many different mosquito species are involved in the transmission of orthobunyaviruses (APPENDIX 1).

Orthobunyaviruses can replicate in mammals and arthropods, but the infection in arthropods is persistent, and no disease occurs. Mammals serve as natural reservoirs or amplifying hosts to orthobunyaviruses. Viruses have been isolated from a large variety of mammals, including bats, rodents, hares, wild birds, sloths, macaques and deer (APPENDIX 1). Reservoir hosts have low levels of antibodies against the virus they carry, and the infection can be persistent for a long time, similar to arthropods, with the host animals remaining healthy. Amplifying hosts serve a template for the virus to amplify and spread further to uninfected vectors. These animals do not remain infected for long, and the animals may develop disease. Because of the wide range of different host animals and vectors, orthobunyaviruses are found all over the world.

Most serogroups are restricted to certain parts of the world. Out of 18 serogroups (and two possibly new serogroups), 9 have been found only in South America. These viruses are found mainly in mosquitoes (Anopheles A and B, Gamboa and Minatitlan serogroups) (Calisher et al. 1980, Lopes Ode et al. 1966, Roca-Garcia 1944, Calisher et al. 1981, Calisher et al. 1983), or their transmission cycle occurs between *Culex sp*. mosquitoes and rodents (Capim, Guama, Group C, Patois and Weyomyia serogroups). The Group C and Guama serogroups include isolates pathogenic to humans (Calisher et al. 1983, Calisher et al. 1983, Causey et al. 1961, Chowdhary et al. 2012) (APPENDIX 1).

The Bwamba and Olifantsvlei serogroups are known to circulate only in Africa. Bwamba virus isolates (Bwamba and Pongola) cause febrile illness in humans, and their distribution areas overlap. Both viruses were isolated from humans decades ago; however, the awareness of these viruses remains low, and clinical cases stay underdiagnosed, although serological studies have shown a high seroprevalence for both viruses (Smithburn, Mahaffy & Paul 1941, Lutwama et al. 2002, Kokernot et al. 1957, Groseth et al. 2014). Several different mosquito species can carry these viruses, but their natural reservoir is

unknown. Olifantsvlei group viruses have been found in mosquitoes only in South Africa, Sudan and Ethiopia (Karabatsos 1985, Ota et al. 1976).

Australian orthobunyaviruses were primarily studied by Doherty et al. when the Koongol and Wongal isolates of the Koongol serogroup were isolated from *Culex annulirostris* mosquitoes in the beginning of the 1960s. In the same study, Mapputta virus (the prototype of the Mapputta serogroup) was recovered from the *Anopheles meraukensis* mosquito. Serological tests showed antibodies in various domestic and wild animals, but no virus isolations were recovered (Doherty et al. 1963). New isolates of the Mapputta serogroup and new unclassified viruses from Australia (Leanyer virus, Termeil virus, Murrumbidgee virus and Salt ash virus) have been later isolated only from mosquitoes in Australia, but the natural reservoir and possible disease associations have remained understudied, although some serological evidence exists (Gauci et al. 2015).

The Bakau serogroup has a narrow range of hosts, and its circulation has been restricted to a small area in the Malaysian forest. Macaques have been identified as the natural host for these serogroup isolates, and their natural cycle involves *Culex sp*. mosquitoes. The only exception is the poorly studied Nola virus isolate, which was isolated from *Culex perfuscus* mosquitoes collected in 1970 in the former Central African Republic (Marchette 1994).

Viruses within the six serogroups are known to be geographically widely distributed. Tete and Turlock serogroup viruses have narrow host ranges, but their distribution area includes all continents except Australia and South America. Migratory and resident birds serve as a host, and none of the viruses have been associated with human disease. Interestingly, Tete viruses use ticks and biting midges as vectors, in contrast to most viruses in the genus (Calisher et al. 1990). The Nyando serogroup includes four species, and each species is found in a different geographical location (APPENDIX 1). Only two of these species - Nyando virus, an African representative causing a febrile illness, and Kaeng Khoi virus, a virus originating from Asia that uses bedbugs as a vector (Williams et al. 1976) - have been officially classified within this serogroup. Recent phylogenetic data suggest that the Brazilian Mojui dos Campos virus (BeAn 2761) and Manéra virus (Eret 147) from Ethiopia also belong to the same serogroup (Groseth et al. 2014). This serogroup also contains viruses isolated from bats, which is a rare exception in genus the *Orthobunyavirus* (APPENDIX 1).

The California, Bunyamwera and Simbu serogroups are the largest serogroups in the genus *Orthobunyavirus* and include the most commonly known pathogens. These serogroups also have the most widespread distribution area, and the viruses use both domestic and wild animals as hosts.

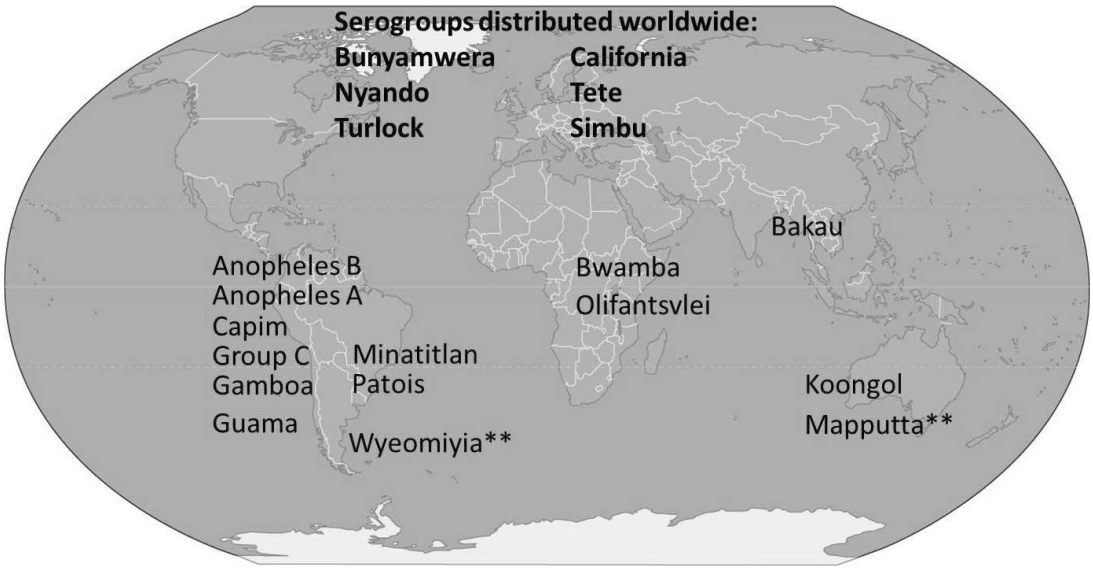

Figure 1. The geographical distribution of orthobunyavirus serogroups (two provisionally unassigned serogroups, Wyeomyia and Mapputta, have been marked with an asterisk). Copyright © 2015 http://www.freeworldmaps.net/

3.2 Orthobunyavirus disease associations in humans and animals

There are many important and well-known veterinary and human pathogens in the genus. The best studied human pathogens of the genus are La Crosse virus (LACV, California serogroup) and Oropouche virus (OROV, Simbu serogroup). LACV is the most common arboviral cause of paediatric CNS disease in the USA, causing 100-200 neuroinvasive infections annually (Reimann et al. 2008). Infections occur primarily in children under 15 years, and the incidence of clinical disease is believed to be 20-30/100 000 inhabitants. LACV infection can vary from non-specific fever to more severe meningitis and/or encephalitis (Haddow, Odoi 2009). OROV has been identified thus far in South America and is the second most frequent cause of arboviral febrile illness in Brazil after Dengue virus infection. Oropouche fever appears most often as large outbreaks, but sporadic human infections also occur. Clinical presentation often includes headache, myalgia, arthralgia, rash and occasional CNS disease (Pinheiro et al. 1981, Bastos Mde et al. 2012). Like other Simbu group viruses, OROV is mainly transmitted by biting midges, particularly *Culicoides paraensis*, but the mosquitoes *Ochlerotatus serratus* and *Culex quinquefasciatus* also play a role in transmitting the virus. The vertebrate host to OROV is sloths (Pinheiro et al. 1981). Tahyna virus (TAHV), a member of the California serogroup, is a cause of "Valice fever" in Central Europe and Asia. It was the second California serogroup virus isolated from humans (Bardos et al. 1975, Simkova, Sluka 1973). Although the California serogroup includes several known human pathogens, the majority of them have been associated with acute infections only by serological evidence (Grimstad et al. 1982, Fauvel et al. 1980). In contrast, many Group C serogroup viruses have been isolated

from humans, but the disease association has remained understudied. Most of these human isolates have been obtained only from South America, where massive mosquito-borne virus sample material collection took place during the 1950-60s, resulting in the identification of 9 new virus isolates (Causey et al. 1961). Several isolates of the bunyamwera serogroup are particularly important in Africa. Ngari virus causes febrile disease and haemorrhagic fever in humans (Gerrard et al. 2004, Briese et al. 2006), and Ilesha virus has been shown to cause febrile illness, erythema, occasional fatal meningoencephalitis and haemorrhagic fever in humans. Both Ngari and Ilesha viruses are reassortant viruses.

Many significant cattle pathogens belong to the Simbu serogroup. Not only Schmallenberg virus (SCHV), which spread rapidly through Europe after 2011 (Hoffmann et al. 2012), but also many other viruses have been associated with similar diseases in ruminants for decades. The symptoms include malformation of the foetus, still birth, abortion and congenital defects. Culicoides midges are the main vectors for viruses in the Simbu serogroup. In particular, Akabane virus (AKAV) is an important pathogen throughout Asia, Australia and some parts of East Africa. It was first isolated in Japan from mosquitoes, but culicoides seem to play a more important role in spreading the virus (Oya et al. 1961). Most Simbu group viruses circulate in Asia and Australia, but a few are found in South and North America and Europe (APPENDIX 1). Especially in Australia, many viruses have been isolated and connected to disease in cattle and small ruminants (APPENDIX 1). Simbu serogroup virus outbreaks cause often major economic losses to farmers. Cache valley virus (CCV) is the BUNMW serogroup virus found in North and Central America. The most likely natural reservoir is in wild ruminants (deer). The virus is found to infect rodents and horses but also, most importantly, domestic ruminants, which suffer from embryonic death, stillbirths, mummification of foetuses, abortions and malformation of foetuses (Chung et al. 1990). Occasionally, CCV infects humans (Sexton et al. 1997, Campbell et al. 2006).

Members of the genus *orthobunyavirus* are well known causes of equine encephalitis or neurologic diseases (APPENDIX 1). Several Bunuamwera serogroup viruses have been isolated from clinically ill horses. Main Drain virus (MDV) is a causative agent of equine encephalomyelitis (Emmons et al. 1983), Maguari virus (MAGV) is a cause of encephalitis (Spence, Downs 1968) and Kairi virus (KRIV) causes febrile illness in horses (Calisher et al. 1988). Several Shuni serogroup viruses are associated with equine infections, especially in Australia, Asia and Africa (Akabane, Douglas, Peaton, Aino, Shuni viruses) (Yang et al. 2008, St George et al. 1979, van Eeden et al. 2012). In addition, Snowshoe hare virus (SSHV), a member of the CEV group, is connected to CNS infections in horses in North America (Lynch, Binnington & Artsob 1985, Heath et al. 1989).

Two orthobunyaviruses have been isolated from pigs. Oya (OYV) and Ingwavuma viruses (INGV) both belong to the Simbu serogroup but are distinct species. Oya virus was isolated from a symptomatic pig during a Nipah virus epidemic in Malaysia. The virus

caused respiratory illness in pigs and was first confused with Nipah virus disease (Kono et al. 2002). Oya virus was also isolated from mosquitoes in Vietnam, and the transmission cycle is believed to occur between mosquitoes and swine (Bryant et al. 2005). Swine are also suspected natural hosts for Ingwavuma virus, as no clear disease is observed in infected swine. Ingwavuma virus was first isolated from *Culex sp*. mosquitoes and the spectacled weaver bird *Hyphanturgus ocularus*. In contrast to Oya virus, Ingwavuna virus antibodies are found in humans, but only in India (Top et al. 1974, McIntosh, McGillivray & Dickinson 1965).

3.3 Molecular biology of orthobunyaviruses

The lengths of the large, medium and small genome segments are approximately 6.9 kb, 4.5 kb and 1 kb, respectively (figure 2). Each segment has a 3'end non-coding region, a coding region and a 5' end non-coding region. The length of the non-coding regions vary within genus; however, the length of the coding region is the same within viruses in the same serogroups (Elliott, Blakqori 2011). There is a unique terminal region at the 3' end (UCAUCACAUG) and at the 5' end (AGUAGUGUGC) in each segment.

The L segment encodes a 259–263 kDa L protein, RNA-dependent RNA polymerase (RdRp), which is able to perform cap snatching of the capped 5' ends of host mRNA (endonuclease) and to use these short nucleotides as primers for viral mRNA synthesis (RNA polymerase). RdRp of orthobunyaviruses is involved in both transcription and replication.

The M segment encodes the 29–41 kDa Gn and 108–120 kDa Gc glycoproteins and a 15–18 kDa NSm non-structural protein. Gc and Gn are integral membrane proteins. Gc shares structural similarities with fusion proteins, but Gn also plays an important role in fusion process (Garry, Garry 2004). The Gc fusion peptide is involved in neurovirulence (Soldan et al. 2010). The antigenic properties of the Gc and Gn glycoproteins are involved in haemagglutination and neutralization. NSm (Fazakerley et al. 1988) protein has been found in all sequenced orthobunyaviruses, and it seems to be involved in virus assembly and budding (Elliott 2014).

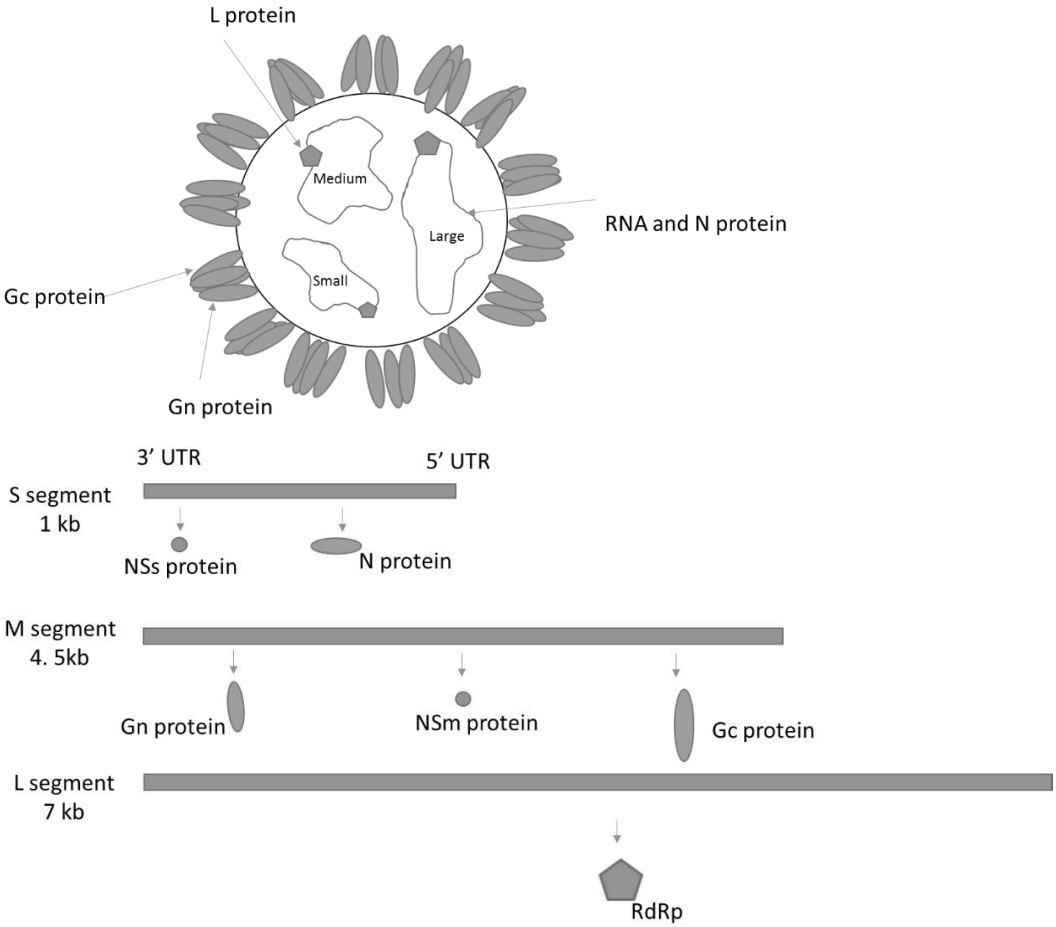

Figure 2. Structure and coding strategy of orthobunyaviruses. Small (S), medium (M) and large (L) segments encode nucleocapsid protein (N), membrane glycoproteins (Gc and Gn), RNA-dependent RNA polymerase (RdRp), and non-structural proteins, NSs and NSm.

The S segment encodes the 19–26 kDa N and 10–13 kDa NSs proteins (King et al. 2012). N proteins are involved in genome encapsidation and in interactions with other viral and host cell proteins (Elliott, Blakqori 2011). They are also associated with complement fixation. The NSs protein inhibits the antiviral interferon system in mammalian hosts and is involved in apoptosis (Blakqori et al. 2007). It also has multiple roles in virus replication and mammalian host cell interaction; it inhibits translation and RNA synthesis (Elliott 2014). However, viruses in the Anopheles A, Anopheles B, and Tete serogroups and Mapputta group viruses have been found to lack the NSs open reading frame (Gauci et al. 2015, Mohamed, McLees & Elliott 2009).

3.4 Genetic reassortment

Reassortments occur within the *Orthobunyavirus* genus, altering the virulence or vector specificity of the virus. Reassortment may occur when closely related viruses circulate in the same area and infect the host at the same time. Segments originally from different viruses end up in the new virus particle during virus assembly. The Bunyamwera serogroup virus Ngari virus (NGIV) is a natural reassortant, in which the S and L segments originate from Bunyamwera virus and the M segment originates from Batai virus. Ngari virus was first isolated from mosquitoes in 1979 and later from human serum samples (Zeller et al. 1996). During a large Rift Valley virus haemorrhagic fever epidemic between 1997-1998, two cases were shown to be caused by Ngari virus (Bowen et al. 2001). The isolates were first named Garissa virus, but later more detailed sequence analysis showed that the Garissa virus isolates were in fact identical to Ngari virus (Gerrard et al. 2004). Bunyamwera and Batai viruses have never been associated with haemorrhagic fevers. (Gerrard et al. 2004, Briese et al. 2006).

Reassortants have been mostly studied in the Bunyamwera serogroup, where, in addition to Ngari virus, Potosi virus (POTV) and Main Drain virus (MDV) are reassortants. Both have Cache Valley virus (CCV) S and L segments and a Kairi virus (KRIV) M segment. Interestingly, Kairi virus has never been isolated in the USA, where Potosi, Main Drain and Cache Valley viruses circulate. The question remains how these viruses have acquired the Kairi virus M segment (Briese, Kapoor & Lipkin 2007). Ilesha virus (ILEV), yet another Bunyamwera group virus, was first isolated in 1959 in Nigeria from a human blood sample. Ilesha virus is a Bunyamwera virus species isolate, but the M segment has a low similarity to all known orthobunyaviruses (Pachler, Ruzek & Nowotny 2013). Because of so many reassortant viruses in the same serogroup, it has been suggested that reassortment may be a driving force in the evolution of this serogroup (Briese, Kapoor & Lipkin 2007). One of the few reassortant viruses outside the Bunyamwera group is the newly identified Itaya virus from Peru. It has only been isolated from two febrile patients and has been shown to have identical S and L segments to Carabaru virus (CAPV); however, the M segment sequence is derived from another still unknown virus (Hontz et al. 2015).

Some orthobunyaviruses have been incorrectly claimed to be reassortants. Schmallenberg virus (SCHV), which emerged in 2011 in Europe, causing severe disease in cattle, sheep and other ruminants, was thought to have evolved from the reassortment of the Sathuperi (SATHV) and Shamonda (SHAV) viruses in the Simbu serogroup (Yanase et al. 2012). However, another group of researchers demonstrated that Schmallenberg virus actually belongs to an old Sathuperi virus species and is possibly the ancestor to this species (Goller et al. 2012). A similar disagreement concerns Guaroa virus (GROV): in serological analysis, Guaroa virus is neutralized by California serogroup virus antibodies, but in complement fixation tests, it seems more similar to the Bunyamwera serogroup viruses (Whitman, Shope 1962). Sequence analysis showed that Guaroa virus is genetically closer to bunyamwera viruses than to California serogroup viruses (Briese,

Rambaut & Lipkin 2004, Dunn, Pritlove & Elliott 1994). However, Guaroa virus is most likely the bridge virus between the CEV and BUNW serogroups (Briese, Rambaut & Lipkin 2004), which in fact was proposed immediately after the initial virus isolation (Whitman, Shope 1962)).

Species- level classification is primarily performed by cross-neutralization and cross-hemagglutination inhibition tests. In addition, according to the International Committee on Taxonomy of Viruses (ICTV), the N protein amino acid sequences of two virus species must differ by more than 10%. Virus isolates within the species can form reassortments, but this does not seem possible between viruses originating from different species. A limitation of the current classification system is that ICTV does not offer any guidance for classifying genotypes, strains or other isolates. In the genus O*rthobunyavirus*, there are many isolates that are insufficiently characterized, virus isolates that lack sequence data or new viruses that may have only sequence data available. Many orthobunyaviruses have been isolated and named, but more data are required to confirm all the isolates as the representative members of the genus.

3.5 Orthobunyaviruses in Europe

There are virus isolates from five different serogroups known to circulate in Europe. The California serogroup is the most common since four of its isolates (Inkoo, Tahyna, Chatanga and Snowshoe hare viruses) have been reported to circulate in Europe. Virus isolates belonging to the Bunyamwera, Simbu, Tete and Turlock serogroups have been recovered from different arthropods and vertebrates as well.

Batai virus (BATV) is the only Bunyamwera serogroup virus found in Europe. The name originates from an isolate found from Malaysia in 1955, but the virus was isolated from three different locations in different parts of the world at the end of the 1950s. A Calovo virus isolate was recovered from mosquitoes from Slovakia in 1960, Chittoor virus was recovered in India in 1957, and Olyka virus was isolated from Ukraine. All of these isolates have been identified as Batai virus strains (Karabatsos 1985). Within Europe, Batai virus circulate mainly in central Europe, but it has been isolated from Sweden and Russia as well (Francy et al. 1989, Jöst et al. 2011). Serological evidence suggests that the virus is also present in Finland (Brummer-Korvenkontio 1973b). Outside Europe, Batai virus has been isolated in many parts of Asia and in Africa (Karabatsos 1985, Nashed, Olson & el-Tigani 1993), where the Batai virus reassortant strains Ngari and Ilesha circulate (Briese et al. 2006). Batai virus seems to infect domestic animals, especially cattle, but until recently, all evidence was based on serological analysis. The first isolates from cattle were obtained in China (Liu et al. 2014). It has been isolated from wild birds, but their importance in the virus lifecycle is not known.

Simbu serogroup viruses were not important in Europe before Schmallenberg virus began to spread in 2011 from Germany and the Netherlands. The virus was found all the way from southern to northern Europe by the end of 2012. Only some eastern parts of Europe did not report any Schmallenberg virus cases. Schmallenberg virus infects not only domestic but also wild ruminants, and the virus is spread by the culicoides biting midges. The natural cycle might include wild ruminants as a reservoir host (Hoffmann et al. 2012, Laloy et al. 2014). Schmallenberg virus is not the only Simbu group virus found from Europe. Although understudied, Ingwavuma virus has been isolated from *Muscicapa striata* birds in Cyprus (Karabatsos 1985).

In addition to Ingwavuna virus, there are several other less well known orthobunyaviruses in Europe that have been isolated from birds. The Tete serogroup Bahig and Matruh viruses circulate in Southern Europe, and both viruses use the *Hyalomma marginatum* tick as a vector and migrating birds/passerine birds as hosts. Both viruses were isolated twice from *Fringilla montifringilla* and *Fringilla coelebs* (Balducci et al. 1973) and share the same distribution area, consisting of Italy and Cyprus (and outside Europe, Egypt). Little is known about the actual distribution and host range of these viruses (Moussa et al. 1974, Hubalek, Rudolf 2012). Lednice virus, in the Turlock serogroup, has a natural cycle between waterfowl and mosquitoes. The virus was isolated in Czechoslovakia from the *Culex modestus* mosquito, which seems to be the only vector for the virus. No vertebrate host is known, and the virus distribution area is restricted to Czechoslovakia and Romania (Karabatsos 1985, Hubalek 2008). Another virus from Czechoslovakia was isolated once from the blood of reed warblers collected in 1984 from Moravia (Hubalek et al. 1990). Sedlec virus (SEDV) was shown to belong to the family *Bunyaviridae* by morphological analysis, but a more detailed analysis of the relationship to other orthobunyaviruses suggested that it forms its own group and is most related to Leanyer virus and the Simbu serogroup. However, only partial sequences have been published, and Sedlec virus remains an unclassified virus within the genus *Orthobunyavirus* (Bakonyi et al. 2013).

4 California serogroup (California encephalitis virus group, CEV group)

4.1 Members of the CEV group

California encephalitis virus (CEV) is considered the only species in the serogroup, which contains 13 virus isolates (Table 1, figure 3.). It was the first isolate in the California serogroup, recovered from *Ochlerotatus melanimon* (*dorsalis*) mosquitoes collected from San Joaquin Valley of California in 1943. The virus causes encephalitis in laboratory mice, rats and hamsters (Hammon, Reeves & Sather 1952). Antibodies were found in humans, horses, cows, rabbits, and ground squirrels, and serological evidence demonstrated that California encephalitis virus can cause encephalitis in humans (Hammon, Reeves 1952). The virus has never been isolated from humans, and only three human cases have been

confirmed. It took 50 years to find the fourth California encephalitis virus case in California (Eldridge et al. 2001), suggesting that various arboviruses should be considered when a patient suffers from a CNS infection.

Dr. Carl Eklund isolated a virus from *Och. trivittatus* mosquitoes in North Dakota and showed its close relationship to California encephalitis virus in cross-neutralization tests. The virus was named Trivittatus virus (TVTV) (Hammon, Reeves & Sather 1952). Trivittatus virus is widespread in the USA and Southern Canada, and human infections occur in all age groups (LeDuc 1987, SUDIA et al. 1971, Monath et al. 1970). A recent study from North Dakota showed that Trivittatus virus was the most common CEV isolate in the area, and its occurrence lasts throughout the summer and until the end of October, in contrast to other CEV viruses. The majority of the isolates have been obtained from *Och. trivittatus* mosquitoes (78%), and some strains still resemble the prototype isolate (Anderson et al. 2015). Phylogenetic analysis of several Trivittatus virus isolates suggest that Trivittatus virus is positioned between the California and Bwamba serogroups, and the divergence at the amino acid level suggests that it is actually distinct enough to be a virus species of its own (Groseth et al. 2015).

The first South American representative, Melao virus (MELV), was isolated from Trinidad in 1955. The virus was isolated from *Ochlerotatus scapularis* mosquitoes, but no human isolates were recovered from 2500 human serum samples obtained from patients with undiagnosed fever within the same area. Only one serum partially neutralized the virus in a very limited serological survey (Spence et al. 1962b). The virus has been identified in areas from Trinidad to Brazil (Carvalho et al. 2009), and antibodies have been found in humans and horses in Argentina, yet no association with clinical disease has been observed (Calisher et al. 1987). Phylogenetic analysis shows that the closest relatives of Melao virus are Keystone and Serra do Navio viruses (Carvalho et al. 2009).

Tahyna virus (TAHV) circulates in an area extending from central Europe to China but is also found in Africa (Kokernot et al. 1962, Lu et al. 2011). Norway is the only country in northern Europe where Tahyna virus has been reported to circulate and was recovered from mosquitoes (*Ochlerotatus sticticus* and *diantaius)* (Traavik, Mehl & Wiger 1978). The African isolate was first named Lumbo virus, but the virus proved to be identical with the Eastern Slovakia Tahyna virus isolate (prototype 92 Bardos). The vertebrate hosts are hares (*Lepus europaeus*), hedgehogs and rodents (Hubalek 2008), and the virus is maintained by horizontal and transovarial transmission in mosquitoes (Traavik, Mehl & Wiger 1978, Bardos, Ryba & Hubalek 1975). Tahyna virus is associated with febrile illness, occasionally causing more severe disease (Bardos et al. 1975, Simkova, Sluka 1973).

Snowshoe hare virus (SSHV) was initially isolated from *Lepus americanus* (snowshoe hare) from Montana and it is common in the northern USA and Canada, where the reservoir hosts *Lepus americanus* and arctic lemmings (*Dicrostonyx torquatus)* are widely distributed (Burgdorfer, Newhouse & Thomas 1961, Ritter, Feltz 1974). There are several

cases of Snowshoe hare virus infection described as encephalitis in humans and horses (Lynch, Binnington & Artsob 1985, Kettyls et al. 1972, Meier-Stephenson et al. 2007). Previously, Snowshoe hare virus was thought to circulate in Russia (Vanlandingham et al. 2002), and some serological evidence supported this finding (Butenko et al. 1991). However, all the strains presented in the Vanlandingham et al. paper (Vanlandingham et al. 2002) appear to have been re-classified as Chatanga virus strains based on NCBI GenBank data, and only one partial Snowshoe hare virus sequence (LEIV-SSH, AF393325) from Russia remains. More studies are required to conclude whether Snowshoe hare virus is actually present in Russia or not.

San Angelo virus (SAV) was first isolated by Grimes et al. in 1958 in Texas from *Anopheles pseudopunctipennis* mosquitoes (Hammon, Sather 1966). In total, 3 isolates were recovered during the 1960s, all from Texas; however, since then, the virus has been largely forgotten. Different mosquito species, including *Psorophora confinnis, Ochlerotatus atlanticus-infirmatus*, and *Anopheles pseudopunctipennis*, are involved in virus maintenance, but no natural host has been identified thus far (Sudia et al. 1971). Low levels of antibodies have been found in white-tailed deer (Issel, Hoff & Trainer 1973). No clinical disease in humans has been identified, and only one confirmed San Angelo virus antibody-positive human individual is known (Karabatsos 1985).

A limited number of studies are available for South river virus (SORV) (NJO-94F), which was isolated from *Anopheles crucians* mosquitoes in New Jersey in 1960. Later virus isolates have been obtained from *Ochlerotatus sollicitans* and *Culiseta melanura* mosquitoes from the same area (Sudia et al. 1971) and from Pennsylvania and Georgia (Wills et al. 1974, Blitvich et al. 2012d). More recently, South River virus was identified as a cause of an infection in horses, sheep and dogs in Mexico (Blitvich et al. 2012c). No evidence of human infection exists.

La Crosse virus (LACV) was initially isolated from the brain tissue of a 4-year-old girl with fatal encephalitis in 1960 (Thompson, Kalfayan & Anslow 1965). She lived in Wisconsin, where the virus-amplifying hosts, chipmunks, were shown to carry the virus. La Crosse virus is mainly transmitted by the tree-hole mosquito, *Ochlerotatus triseriatus* (Gauld et al. 1975, Thompson et al. 1972), and the virus is maintained in nature by transovarial and horizontal transmission cycles between vectors and hosts (Watts et al. 1973). Aedes triseriatus hatch late in the spring, which results in the appearance of La Crosse virus encephalitis cases as a sharp peak later in the summer, typically between July and September. It circulates in the Appalachian and Midwestern regions of the USA (Haddow, Odoi 2009)and was isolated from Tennessee from non-native *Aedes albopictus* mosquitoes in 1991 (Gerhardt et al. 2001). This finding suggests that new vector species for La Crosse virus may affect the virus distribution and the number of clinical cases could increase.

Jamestown canyon virus (JCV) was isolated for the first time from *Culiseta inornata* collected from Colorado in 1961 (DeFoliart et al. 1969), and since then, the virus has been isolated from most of the USA and Canada (Fulhorst et al. 1996, Boromisa, Greyson 1990, Srihongse, Greyson & Deibel 1984). Boreal mosquito species are the principal vectors, but the virus is also carried by deer and horse flies (DeFoliart et al. 1969, Andreadis et al. 2008). The natural cycle involves white-tailed deer and other wild ruminants as an amplification host (Issel, Hoff & Trainer 1973, Zamparo et al. 1997). Although the deer populations have increased recent years, it has not affected the Jamestown canyon virus prevalence in mosquitoes, indicating that transovarial transmission is important for virus survival (Anderson et al. 2015). Humans are a dead-end host to the virus, and the symptoms of the disease may vary from mild febrile illness to encephalitis (Pastula et al. 2015).

Keystone viruses (KEYV) circulate in the south and southeast regions of the USA. Many hundreds of isolates have been recovered from mosquitoes, mostly from *Ochlerotatus atlanticus* mosquitoes between 1943 and 1970. Almost 95% of all Keystone virus isolates have been recovered from *Och. infirmatus* and *Och. taeniorhynchus* (Sudia et al. 1971). The virus life cycle probably involves cotton rats, the distribution of which is restricted to southeastern USA (Taylor et al. 1971). Transovarial transmission has been shown to be important in virus maintenance and transmission, as the virus has been isolated several times from mosquito larvae and male mosquitoes (LeDuc et al. 1975). Keystone virus can infect humans, but clinical disease from this virus has not been observed. Studies show a 16-22% antibody prevalence among the human populations studied (Parkin, Hammon & Sather 1972).

Inkoo virus (INKV) is one of the European CEV representatives, and its distribution is limited to northern Europe, Finland, Sweden, Norway and Russia (Brummer-Korvenkontio et al. 1973, Niklasson et al. 1985, Traavik, Mehl & Wiger 1978, Butenko et al. 1991). INKV was found in 1964 from *Ochlerotatus communis/punctor* mosquitoes. Antibodies have been found in many vertebrates (Brummer-Korvenkontio 1973a), but the natural reservoir remains unknown. It has been speculated that moose or deer take part in the natural cycle of the virus. *Lepus timidus* populations also have a high INKV seroprevalence. INKV seems common in Northern Europe, where the seroprevalence among humans is 20-40% (Niklasson et al. 1985, Brummer-Korvenkontio 1973a, Traavik, Mehl & Wiger 1985), but no clinical disease has been reported yet. Interestingly, INKV is phylogenetically closely related to Jamestown canyon virus (96,6% S segment aa identity), although the geographical distribution of these viruses is widespread (Vapalahti et al. 1996).

The second South American representative, Serra do Navio virus (SDNV), was isolated from *Ochlerotatus fulvus* collected in Amaba, Brazil in 1966. No other virus isolates have been recovered, and although antibodies were found in humans, spiny rats and opossums, there is no association with any disease. The natural life cycle of the virus is

not known (LeDuc 1987, Karabatsos 1985). Phylogenetic studies have shown that the virus is most closely related to Inkoo, Jamestown canyon, South River and Keystone viruses (Huang et al. 1996).

Chatanga virus (CHATV) isolates were first obtained from mosquitoes collected in Murmansk, Russia in 1983. Several isolates were recovered from mosquitoes collected all over Russia between 1983 and 1989. The distribution area covers all of Russia and eastern parts of Finland. It overlaps with Inkoo and Tahyna virus distribution in western parts of Russia (Lavrent'ev et al. 2008). There are no data available on the host range and disease association of CHATV. CHATV is closely related to Snowshoe hare and La Crosse virus in phylogenetic analysis (Putkuri et al. 2014).

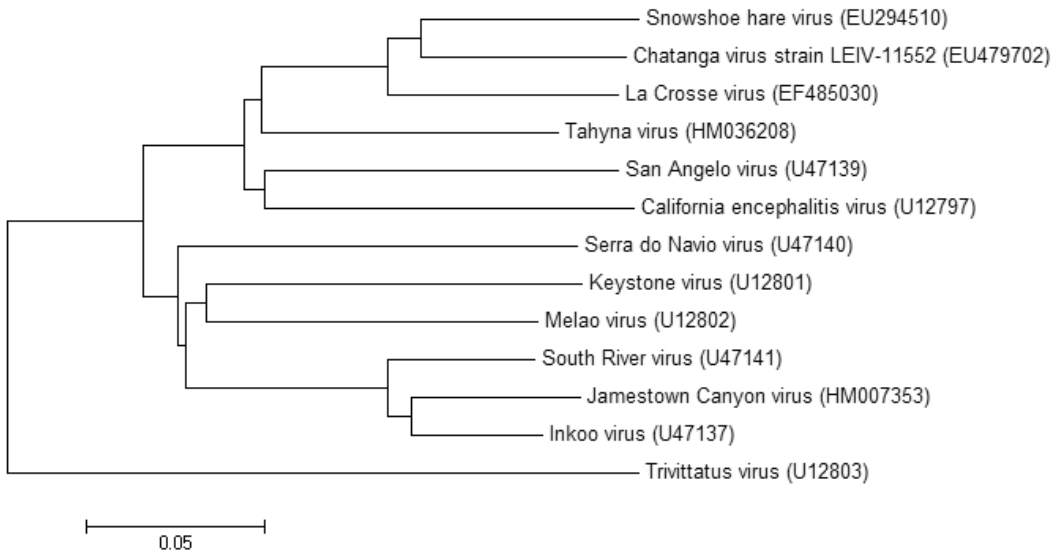

Figure 3. Neighbour-joining phylogenetic tree of the S segment of California serogroup viruses.

4.2 Transmission cycle of the CEV group

All CEV group viruses have a unique transmission cycle, using vertebrate hosts to persist in nature (APPENDIX 1). Humans are not an essential part of the transmission cycle, and they are referred to as dead end hosts since the plasma viremia level remains too low to infect new mosquito vectors. Viruses may have several different host cycles (Gauld et al. 1975, Ksiazek, Yuill 1977, Goff, Whitney & Drebot 2012), and the vector species transmitting the virus may differ in each cycle. The maintenance of these cycles is dependent on the transmission of the virus to the next generation of mosquitoes (or eggs) and to new uninfected hosts.

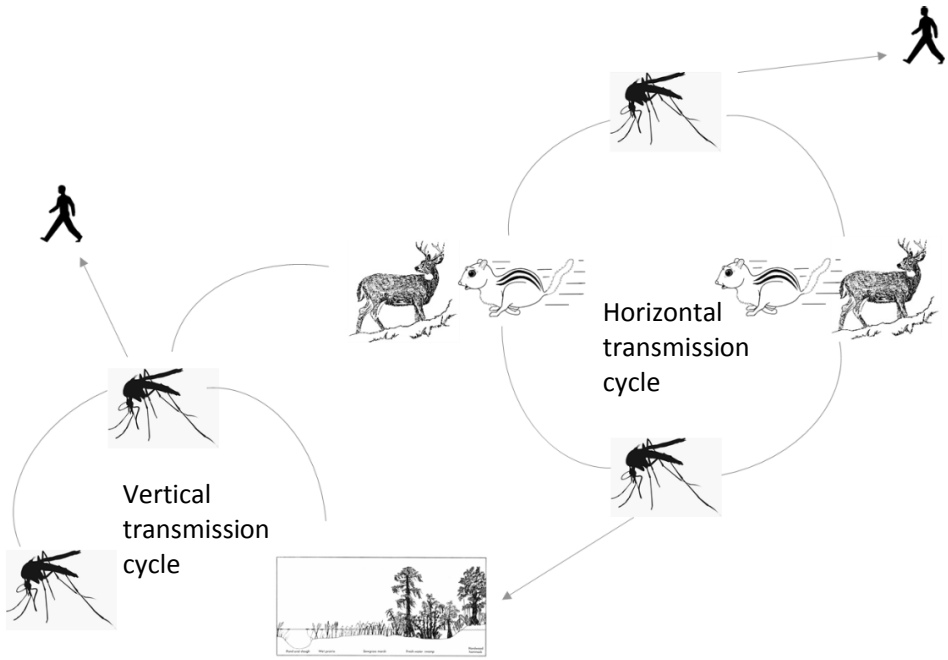

Figure 4. The La Crosse virus natural cycle involves chipmunks, deer and the tree-hole mosquito *Aedes triseriatus*. The figure demonstrates the horizontal and vertical transmission route for La Crosse virus. Modified from (Reisen 2002, Borucki et al. 2002).

The predominant way for transmission for the CEV group is horizontal maintenance, which involves vector-host cycles, or mating between infected male and uninfected female mosquitoes (venereal transmission) (figure 4) (Thompson, Beaty 1977). The plasma viremia is brief in the host animals; for example, viremia of La Crosse virus-infected chipmunks is high enough to infect mosquitoes for 2 to 4 days only. However, experimental infections have shown that the majority of the mosquitoes become infected after a blood meal during viremia (Pantuwatana et al. 1972). However, venereal transmission is considered to be at least as important method of virus maintenance. The venereal transmission efficiency is somewhat influenced by the timing of the female blood meal. Blood meal consumption before mating enhances the likelihood of venereal infection (Thompson 1983). It has been shown that mosquitoes are more eager to mate if they carry the virus (Reese et al. 2009) and are also more motivated to obtain the blood meal if the vector is infected with the virus (Jackson, Brewster & Paulson 2012)

Several viruses in the CEV group use vertical transmission maintenance, where the virus is transmitted from infected females to progeny (transovarial transmission)(Watts et al. 1973, LeDuc et al. 1975). La Crosse, Trivittatus, Jamestown canyon, Snowshoe hare and Keystone viruses have been isolated from mosquito larvae and male mosquitoes, which demonstrates that the virus is transmitted into mosquito eggs by transovarial transmission (Anderson et al. 2015, Watts et al. 1973, LeDuc et al. 1975, Mclintock et al.

1976, Andrews et al. 1977). This serves as an additional winter-season reservoir for viruses and enables the virus to resume the transmission cycle early in the spring (Watts et al. 1974). Interestingly, transovarial transmission seems to be a more important feature for mosquitoes in the north, where the number of infected progeny is higher than similar mosquito species in south. On the other hand, the number of viable eggs were lower if they carried the virus (Woodring et al. 1998).

The timing of a possible disease peak in the human population during the mosquito season depends on the method of virus transmission (Anderson et al. 2015). If horizontal transmission is the primary method to amplify the virus, more infections occur later in the summer/early autumn, when the virus prevalence is higher in mosquito vectors (Cache Valley Virus). Vertically transmitted viruses primarily cause a disease peak early in the season. If both methods are used to transmit the virus (e.g., Jamestown Canyon virus), infections may be found throughout the mosquito season (figure 5).

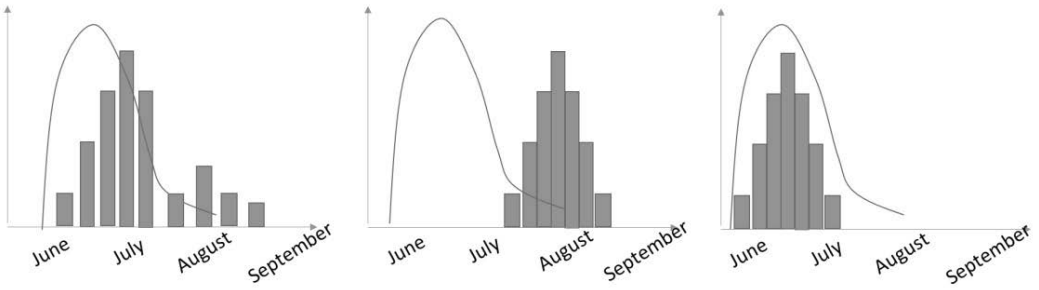

Figure 5. Examples of virus maintenance and the influence of the route of transmission on the amount of virus present during the summer months. Modified from (Anderson et al. 2015). a) The pattern if the virus uses vertical and horizontal transmission in maintenance, b) the pattern if horizontal transmission only is used and c) the pattern if vertical transmission only is used. Columns show the amount of virus isolated, and the curve shows the amount of mosquitoes present.

The presence of antibodies in wild mammals is not direct evidence that the animals have developed a sufficient level of viremia to infect vectors. Experimental studies on hosts for La Crosse virus have shown that the virus titre and the duration of the sufficient titre may vary between host species. In chipmunks, the virus titre stays high enough for 3 days, whereas in foxes, the titre never reaches the same level, yet the viremia lasts longer (Yuill 1983). Interestingly, the predators (foxes) could become infected by eating the prey (chipmunks), but the infection was not acquired while drinking water with the mosquito larvae carrying the virus (Yuill 1983, Amundson, Yuill 1981).

4.3 CEV replication

CEV enters cells by receptor-mediated endocytosis (Hollidge et al. 2012). Glycoprotein Gc is more important in vertebrate cell attachment, and Gn is more important in arthropod cell attachment (Ludwig et al. 1991). However, other studies show that Gc is involved in infecting both cell types (Hacker, Hardy 1997). The receptor involved in virus-cell attachment is unknown. La Crosse virus uses clathrin-mediated endocytosis to enter mammalian cells, but other CEV viruses are less well studied. Endosomal trafficking transports the virus through the plasma membrane and protects from immune surveillance. This route also enables the virus to enter the primary neurons, which may lead to CNS infection. After entry, the virus is transported in endocytic vesicles into the early endosomes, and this transport is regulated by the Rab5 GTPase (Hollidge et al. 2012). Low pH in the early endosome is required for the uncoating of the virus and fusion of the viral membrane with the endosomal membrane (Gonzalez-Scarano, Pobjecky & Nathanson 1984). Gc is involved not only in cell attachment but also in membrane fusion (Plassmeyer et al. 2007).

After uncoating, the nucleocapsids are released into the cytoplasm, where replication takes place. Genomic RNA is transcribed into positive-sense mRNA by RdRp (primary transcription). The L protein of La Crosse virus contains a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease. Primary transcription is primer-dependent, and RdRp uses nucleotides originating from the host as primers to transcribe the viral mRNA. The primers are host mRNAs that have been cleaved from the 5' end by viral RdRp in a process called cap snatching and hence lack the capped oligonucleotides (Patterson, Holloway & Kolakofsky 1984). Translation of viral proteins from mRNA is required for transcription to occur. The on-going protein synthesis in host cell ribosomes guarantees the production of full-length viral mRNAs (premature termination of transcription) (Bellocq, Kolakofsky 1987).

The switch from transcription to genome replication happens when a sufficient level of N protein is available in the cell for encapsidation. Only the L and N proteins are needed for replication. Genomic viral RNA is used to produce anti-genome as a template for replication. The produced RNA segments are encapsidated by the N protein and associated with the L protein, forming nucleocapsids (RNPs) that appear circular in the virion and in infected cells (Elliott 2014). N protein encapsidates both genomic and antigenomic RNA to inhibit the formation of dsRNA and prevent the host immune response and interaction with transcription (Ogg, Patterson 2007).

The virus particles assemble and mature by budding from the Golgi apparatus. The Gn and Gc glycoproteins accumulate at the Golgi complex membrane after glycosylation in the ER. Nucleocapsids are transported to the modified Golgi membrane and bud through into the Golgi membrane-derived vesicle. Maturation of the virus particle happens during transport from the Golgi stacks to the trans-Golgi. This changes the glycoproteins Gn and Gc and forms intermediate particles (Madoff, Lenard 1982). The particles are transported from the Golgi to the cell surface. Fusion of the particle membrane with the plasma membrane leads to the release of infectious virions (Elliott 2014).

4.4 CEV pathogenicity

The viral infectivity and virulence of CEV has been mostly studied with La Crosse virus (LACV) and Tahyna virus (TAHV) in mice. Susceptibility to infection is dependent on the age of the host; young hosts suffer from more severe infection than adults (Johnson 1983). It seems that virus strains that cause encephalitis in mice after peripheral inoculation can cause encephalitis in humans (Tignor et al. 1983). In mice, LACV infects peripheral tissues near the inoculation site, where the initial virus replication happens (Taylor, Peterson 2014). The virus enters the bloodstream after 24 hours, causing plasma viremia. However, the virus can already be found replicating in the brain after 6 hours of infection because of its spread via the subarachnoid space into the brain (Johnson 1983). It has been suggested that LACV presumably gains access to the CNS via olfactory neurons (Bennett et al. 2008). Plasma viremia leads to infection in the muscles and brown fat. Orthobunyaviruses establish plasma viremia rapidly in order to be transferred to new insect vectors. Viruses use the NSs protein to overcome the fast-responding innate immune defence of the mammalian host (Blakqori et al. 2007). Viremia lasts 1-3 days, and at the end, the infection is widespread and the virus is present in most of the tissues (skeletal, cardiac and smooth muscles and their small arteries and veins) (Johnson 1983). During plasma viremia, a high level of replication happens in the respiratory tissue as well, and CNS infection appears subsequently. The highest level of viremia during the infection is found in the nasal turbinates, brain and spinal cord. Infection of adult mice rarely leads to clinical disease (Bennett et al. 2008).

Inoculation of rhesus monkeys with LACV, TAHV and Jamestown canyon virus (JCV) did not lead to clinical disease. All viruses produced a strong neutralizing antibody response that was first detectable 6 to 8 days after infection. Plasma viremia was not detectable after TAHV and LACV infections; however, JCV caused viremia 2 to 4 days after infection (Bennett et al. 2008, Bennett et al. 2011a, Bennett et al. 2011b). LACV was found in the lymph nodes, but replication could not be detected (Bennett et al. 2008). The level of plasma viremia seems to correlate with the neutralizing antibody levels, and non-human primates seem to mimic the infection in humans, with a low incidence of clinical disease (Bennett et al. 2011a).

The virus infection is not harmful to the invertebrate vector and leads to persistent infection. Viruses infect and begin replicating in the midgut epithelial cells of the mosquito within a few hours after the blood meal. During the next 5 to 6 days, infection is not apparent and not even detectable; this is referred to as an eclipse phase. A few days later, the virus particles pass through from the gut epithelial cells into the haemocoel, which enables the particles to spread further and infect various organs. The salivary glands are the last tissues to be infected (Tesh, Beaty 1983). After replicating in the salivary glands, the glandular lumen is packed with virus particles, and mosquitoes are ready to transfer the virus into a new uninfected animal host. The time period from initial infection until the vector is ready to spread the virus is 7-14 days for California encephalitis virus (Watts, DeFoliart & Yuill 1976, Chernesky 1968). Transovarial transmission can happen after the virus has reached the mosquito ovaries, which means

that after the initial blood meal, a mosquito's first batch of eggs is virus-free. It is not known how the virus enters the eggs. However, transovarially infected mosquitoes are instantly ready to spread the virus (Miller, DeFoliart & Yuill 1979).

The mechanism of persistent infection in invertebrates is not well known. Insects do not develop antibodies against the virus; instead, they use the RNAi response for the specific recognition and destruction of viral-RNA (Sanchez-Vargas et al. 2004). La Crosse virus infection is known to cause a strong RNAi response in infected insect cells. However, in contrast to mammalian host infections, the anti-RNAi activity of the NSs protein to prevent the RNAi response is not observed in mosquitoes (Blakqori et al. 2007). Orthobunyaviruses replicate rapidly, and new infectious viruses are released from the cell without rupturing the endomembranes of the cell (Golgi stacks). At the same time, the viral proteins seem to aggregate into large complexes, which helps the virus to escape the cell response. Additionally, morphological changes are observed in mosquito cells (Lopez-Montero, Risco 2011). Noncytopathic persistent infection in mosquito cells has been suggested to occur because of the reduction of the synthesis of the host mRNAs, which are used as transcriptional primers for viral synthesis. In addition, the virus appears to self-limit the production of the N protein by encapsidation of S segment-derived mRNA, which means that the switch from transcription to genome replication does not happen (Hacker, Raju & Kolakofsky 1989).

4.5 Clinical disease in humans

Only two of the California serogroup viruses have been isolated from humans thus far; TAHV and LACV. TAHV circulates in central Europe, and the human isolates have been obtained from sick children living in southern Moravia in the former Czechoslovakia. The first patient had fever, conjunctivitis and pharyngitis two days after the onset of symptoms. The second child showed meningeal symptoms with fever and sore throat one day after falling ill. Both patients were under 10 years old, and the virus was isolated from blood samples (Bardos et al. 1975). Both patients were treated in Valtice hospital, which designated TAHV disease as Valtice fever. The clinical picture of Valtice fever is most often similar to influenza-like or pulmonary disease (70%). In fewer cases, the patients suffer from abdominal or rheumatic disease (20%), tonsillitis (7%) or CNS symptoms (3%). One study presented cases over a 4-year period and included 58 patients, mostly male (65%). The incubation period observed for the TAHV disease was 3-4 days, and the disease was not connected to a specific age group (Sluka 1969).

Several large retrospective studies have been published of the association of LACV with encephalitis in children (Balfour et al. 1973, Haddow, Odoi 2009, McJunkin et al. 2001). Most patients were under 15 years old (83%), but adults also became infected (Haddow, Odoi 2009). In the studies mentioned above, 60% to 70% of those infected were males. Fever and headache were the most prevalent symptoms (70-80%) in addition to altered consciousness, gastrointestinal symptoms and seizures (Balfour et al. 1973, McJunkin et al. 2001). The cases occurred from July to September. LACV infections have been divided into mild and severe forms. The mild form usually presents with headache, fever and

gastrointestinal symptoms, whereas the more severe forms manifests with meningoencephalitis, encephalitis or meningitis (Balfour et al. 1973, Haddow, Odoi 2009). Severe cases have become more frequent over the years (Haddow, Odoi 2009); however, the virus is rarely isolated from patients with CNS involvement (McJunkin et al. 2001). The case fatality rate for human LACV infections is 1.9% (Haddow, Odoi 2009), and the virus isolates from fatal cases belong to a very narrow range of virus isolates that circulate mainly in Midwestern USA (Huang et al. 1997, Lambert et al. 2015).

Jamestown Canyon virus (JCV) infections have been underdiagnosed for years in the USA; the number of diagnosed cases increased by 50% due to the implementation of specific IgM tests for JCV at the CDC in 2013 (Pastula et al. 2015). CNS involvement is found in up to 50% of cases, but many studies have concentrated on patients with neuroinvasive disease. Compared to LACV infection, JCV is more prevalent in adults than in children (Grimstad et al. 1982, Pastula et al. 2015, Grimstad et al. 1986). JCV is neuropathogenic, but the symptoms are less severe than those in LACV infection in children. Most of the cases are diagnosed from July to September, yet the disease peak is not as high as those of other arboviral diseases. JCV infections occur throughout summer because the so-called early season mosquitoes are involved in virus transmission (transovarial transmission to mosquito eggs) and may infect humans early in the summer (Pastula et al. 2015). In addition, different mosquito species transmit the virus at different times of the summer (Andreadis et al. 2008). The average patient is a 40-59-year-old male, and typical CNS involvement includes meningoencephalitis and meningitis (Srihongse, Greyson & Deibel 1984, Pastula et al. 2015, Centers for Disease Control and Prevention (CDC) 2011).

Snowshoe hare virus (SSHV) is the most frequent in Canada, where one symptomatic infection is recorded every year (Meier-Stephenson et al. 2007). Several cases of SSHV encephalitis have been published, and all of these cases have been confirmed with serological tests from the paired serum samples. Typically, patients with SSHV encephalitis are children under 11 years old, and the symptoms are similar to those of LACV and JCV infections (fever, headache, vomiting) (Fauvel et al. 1980, Embil, Camfield & Artsob 1985, Embil et al. 1982). The patients seem to recover rapidly; they are discharged from the hospital within one week, and all have recovered completely. The antibody levels rise quickly, indicating short-lasting viremia, which might be responsible for the poor success of virus isolation from infected humans (Fauvel et al. 1980, Meier-Stephenson et al. 2007). Most published cases have been diagnosed in August.

California encephalitis virus was identified as a cause of encephalitis two years after the discovery of the virus (Hammon, Reeves 1952), but it took more than 50 years to find the next case of California encephalitis (Eldridge et al. 2001).

Infections in humans caused by the rest of the CEV group viruses have been inadequately described. Trivittatus virus antibodies have been found in all age groups in the USA, but the seroprevalence seems higher in the elderly (Monath et al. 1970). The situation is similar to that of the human Inkoo virus antibody prevalence in northern Europe (Brummer-Korvenkontio 1973a, Traavik, Mehl & Wiger 1985). Keystone virus antibodies

have been found only in residents of Louisiana and Florida (Parkin, Hammon & Sather 1972), whereas there is less evidence of Melao and Derra do Navio virus infections in humans. Evidence of Melao infection in humans is based on a single serum sample from Trinidad (Spence et al. 1962b) and two positive samples out of 20 from Argentina (Calisher et al. 1987). Serra de Navio virus antibodies have been found in humans in Amaba, where the virus was also originally isolated (Karabatsos 1985).

4.6 California serogroup viruses in Northern Europe

The geographical distribution of Inkoo virus (INKV) is limited to Northern Europe (Brummer-Korvenkontio et al. 1973, Francy et al. 1989). The prototype strain (KN3641) was isolated from *Ochlerotatus (Aedes) communis/punctor* mosquitoes collected in a municipality in south Finland in 1964 (Brummer-Korvenkontio et al. 1973). The virus was identified with cross-complement fixation, cross-haemagglutination inhibition and cross-neutralization tests as a member of the California serogroup within orthobunyaviruses. In addition, electron microscopy was used to study the morphology of the virus. To date, three different Inkoo virus strains have been isolated from Finland; two from the place called Inkoo and one from the nearby municipality Kirkkonummi. The second isolate from Inkoo (KN5213) was recovered from *Aedes* mosquitoes collected one month after the prototype strain. The third isolate (KN8110) was recovered from a pool of *Aedes* and *Culiseta* mosquitoes collected 2 years later (Brummer-Korvenkontio et al. 1973). The isolations were carried out in suckling mice. Inkoo virus antibodies were found in humans, cows, reindeer and wildlife (moose, foxes, *Lepus timidus* hares), and the antibody prevalence was higher in northern Finland (70%) than in the south (11%) (Brummer-Korvenkontio 1973a). In phylogenetic analysis, INKV was most closely related to Jamestown Canyon virus, which is known to circulate in the USA only (Vapalahti et al. 1996). The disease association of INKV infection has been poorly characterized, although some evidence for human disease has been reported in the Russian scientific literature (Demikhov, Chaitsev 1995, Demikhov 1995).

Since the isolation of INKV in Finland, the virus has also been recovered from Sweden, Norway and western Russia (west of the Ural Mountains). The isolates have originated from *Ochlerotatus communis* collected from central Sweden, near Sundvall and Eddsbyn (Niklasson et al. 1985, Francy et al. 1989), *Ochlerotatus diantaeus* from the town of Masi in northern Norway (Traavik, Mehl & Wiger 1978) and *Och. communis*, *Och. punctor* and *Ae. flavescens* mosquitoes from a widely distributed geographical area in Russia (Butenko et al. 1991, Mitchell et al. 1993). The isolate from Norway was recovered from a male mosquito, indicating the ability of INKV to be transovarially transmitted. The recently isolated INKV strain Lovanger RNA from *Ochlerotatus communis* larvae from Sweden supports this finding (Tingström et al. 2016). Interestingly, the Norwegian isolate has never been sequenced, and its characterization was based on serological comparison to TAHV. It had a less close relationship with TAHV than did the two other tested isolates. Norway is the only country in northern Europe where Tahyna virus has been shown to circulate (Traavik, Mehl & Wiger 1985).

4.7 Diagnostic methods for CEV infections

Diagnosing California serogroup virus infection is still mainly accomplished by serological testing. The strains in the California serogroup are closely related but antigenically distinct. This poses a challenge for serodiagnostic testing and makes neutralization tests (NT) still the most reliable method to identify the cause of a CEV infection. Historically, haemagglutination inhibition (HI) and complement fixation (CF) tests were used in serological screening (Clarke, Casals 1958, Chappell et al. 1969), and paired serum samples were requested to confirm the result. However, it has been shown that CF is not useful for rapid diagnosis; CF may take almost three times longer after the onset of symptoms to demonstrate seroconversion between paired samples than HI and NT tests (Calisher, Bailey 1981). Several less laborious approaches have been published and used in diagnostic laboratories, such as indirect fluorescence assays (IFAs) and enzyme immunoassays (EIAs). IFA tests have proven to be sensitive enough, and antibodies are detected soon after the onset of symptoms (Beaty et al. 1982). EIA/ELISA tests are most often targeted to detect IgM antibodies and used for testing acute phase samples. Both IFA and EIA/ELISA methods are useful for the rapid primary serodiagnosis of CEV infection but require NT as a confirmatory test because different virus strains cross-react in these tests (Calisher et al. 1986) .

There are increasing numbers of nucleic acid amplification systems available, and many of the PCR systems are designed to find California serogroup virus RNA from mosquito samples. The S segment is the most usual genomic region targeted (Kuno et al. 1996, Lambert et al. 2005). These methods also work when detecting RNA from human tissue samples, and PCR systems have replaced old-fashioned virus isolations and suckling mouse experiments. However, little is known about their usability to detect RNA from serum and CSF since the viremia caused by California serogroup viruses is short and the viral load is low in humans. The virus is not typically able to cross the blood-brain barrier.

5 Aim of the study

Inkoo virus has been known to circulate in Finland for several decades, yet the awareness of the virus among the physicians is poor, and no modern diagnostic tools to detect the infection exist. The possible disease associations have not been systematically studied, although the virus is known to infect humans, and closely related isolates from the same serogroup have been associated with encephalitis.

In 1974, in the doctoral thesis of Markus Brummer-Korvenkontio, it was suggested that another California serogroup virus was circulating in Finland based on conflicting results from the serological analysis of hare samples. However, no attempts to isolate the virus from mosquitoes have been conducted during the last few decades.

The specific aims of the thesis were:

- Develop diagnostic methods for the detection of Inkoo virus and other California serogroup virus infections

- Study Inkoo virus and California encephalitis virus seroprevalence in humans in Northern Europe and to determine the frequency of acute infections

- Characterize the clinical picture of Inkoo virus infections in humans

- Isolate California serogroup viruses from Finnish mosquitoes

6 Materials and methods

6.1 Samples

6.1.1 Patient serum samples (original publications I, III, IV)

All patient serum samples collected in Finland are shown in the original publication III in table 1. Suspected Puumala virus infection samples (n=1294) from 2001 were used for INKV seroprevalence studies and the evaluation of new serological methods. All samples were used to find acute Californian serogroup virus infections. All samples were originally sent to Helsinki University Central Hospital laboratory when Puumala or Inkoo virus infections were suspected or when the patients were screened for causative agents of neurological infections. In addition, two previously confirmed INKV cases were included in the study of the characterization of the INKV clinical picture. A panel of CSF samples (n=832) was tested for INKV IgM antibodies.

To study INKV seroprevalence in Northern Sweden, a total of 1729 serum samples were screened. The study material was part of the WHO MONICA Study (Eriksson et al. 2011), which was a population-based survey and included randomly selected subjects between 25-74 years of age selected from population registers and was stratified for age and gender. The samples used for INKV seroprevalence screening were collected from January to April, 2009.

6.1.2 Mosquitoes (original publication II)

A summary of the mosquito collections is presented in table 2. The mosquitoes were collected using hand nets (human bait) or a BG-sentinel mosquito trap system (luring substance bait) both in the morning and in the evening in wetland areas. Traps were also sometimes left to collect overnight. The collected mosquitoes were briefly frozen alive at -20 degrees, tubed and stored on dry ice before long-term storage in a -70 freezer. Before isolation attempts, the mosquitoes were pooled in 10 mosquitoes per pool and pulverized with a pestle in Dulbecco's 0.2% BSA.

Table 2. Summary of mosquito collection between 2004 and 2009. Number of collected mosquitoes, collection time and place are presented in the table.

Year	Place	Time	Number of Individuals
2004	Rääkkylä, East Finland	25.-27.6.	537
	Konnevesi, Central Finland	7.-9.7.	1870
	Lammi, South Finland	24.8.	1460
2005	Mekrijärvi, (Ilomantsi), East Finland	1.-3.7. 23.-25.8.	1639 210
	Tvärminne, South Finland	8.7. 19.8.	740 360
2006	Mekrijärvi, East Finland	29.6.-1.7.	1639
2007	Sotkamo, Kainuu	21.8.-22.8.	800
	Mekrijärvi, East Finland	23.8.-25.8.	560
2008	Mekrijärvi, East Finland	22.-24.8.	1639
2009	Inkoo, South Finland	14.9.	90
	Mekrijärvi, East Finland	17.-19.8.	1500
			11,430 mosquitoes and 670 mosquito larvae

6.2 Serological methods (original publications I, III and IV)

IFA (I, III, IV)

Vero E6 cells were grown in 75 cm^2 cell culture bottles (Greinerbio-one, Germany) and were infected with the selected virus when 80-90% confluent. The cell monolayers were washed with PBS and incubated at 37°C for one hour with 400 µl of virus suspension (final concentration 500-1000 pfu per 1 ml) before adding 20 ml Eagle's minimal essential medium (MEM) media. The cells were harvested with trypsin-EDTA after 24 hours. The cells were washed 3 times with PBS before suspending in 7 ml of the final concentration, and 20 µl were added to each well on slides (Immuno-cell). The slides were air dried and fixed with acetone for 7 minutes.

An IFA test was used to screen Inkoo virus IgG and IgM antibodies from human serum samples. In addition, California, Bunyamwera and Simbu serogroup IgG antibodies from hare, dog, rodent and horse whole blood and serum samples were screened with an IFA test. IgG and IgM tests were performed by incubating the serum/blood dilutions at 37°C for one and four hours, respectively. The slides were washed with cold PBS, stained with fluorescein isothiocyanate-conjugated anti-human IgG or IgM (Jackson ImmunoResearch laboratories) for 30 minutes at 37°C and washed with PBS before examining with a fluorescence microscope.

CPE neutralization tests (I, IV)

A mini-scale neutralization test was performed in 96-well plates, where Vero E6 cells were grown to full confluence. Selected viruses were diluted to optimal concentrations (500 to 1,000 PFU per ml), and serum samples were first inactivated for 10 min in 50°C

and then serially twofold diluted starting from 1:20. Serum dilutions and diluted viruses were combined in a 1:1 ratio, incubated for 1 h at +37°C, and then added to the cells for 1 h. The cells were inoculated with the virus-serum suspensions and observed after 2 days to detect CPE. The neutralization titre was defined as the final serum dilution that completely inhibited the cytopathogenic effect (CPE). A CPE neutralization test was used to screen California, Bunyamwera and Simbu serogroup antibodies from deer blood samples.

Plaque reduction neutralization test (PRNT) (III, IV)

PRNT test protocol followed that described for the CPE neutralization test. The only exception is that Vero E6 cells were grown on 6-well plates to full confluence. After both incubations, the virus serum dilution suspensions were removed before 3 ml cell media (EMEM) agar solution was added containing a 1% low-melting-point agarose, antibiotics, glutamine and 10% FBS. The plaques were counted after 3–7 days by crystal violet staining. The titre was defined as the final dilution that reduced 60% of the plaques. PRNT was used to confirm the IFA and CPE neutralization results.

6.3 Virus isolation attempts (original publications II and III)

For isolation attempts from mosquito samples, green monkey kidney cells (Vero cells, ATCC CCL-81™) and *Aedes albopictus* clone C6/36 cells were used for cell-culture screening, while for human serum samples, Vero E6 cells (CRL-1586™) were used. Both Vero cell lines were grown at 5% CO_2 at 37°C, and C6/36 cells were grown at room temperature to 80–90% confluence in a cell monolayer in 25 cm^2 flasks.

For infection, 200 µl of the unfiltered mosquito suspension was added to Vero cells, and filtrated suspension was added to C6/36 cells. One hundred microliters of serum samples were used to infect Vero E6 cells. The samples were incubated on cells under cell culture conditions for 1 hour before media was added. The media used for both Vero cells was EMEM with foetal bovine serum to a final concentration of 2% and glutamine, penicillin, and streptomycin. C6/36 cells were grown on L-15 media with 2% foetal bovine serum and antibiotics. The media were changed after one day, and the cells were observed daily for the occurrence of (CPE) during a two-week cultivation period. After two weeks, if no CPE was observed earlier, 3 aliquots of supernatant were stored at -70°C, and the cells were harvested to produce IFA slides from all samples.

IFA slides were prepared as described earlier and stained using a selected panel of antisera of the genera Alphavirus (Sindbis-virus), Flavivirus and Orthobunyavirus (California serogroup (Tahyna polyclonal) and bunyamwera serogroup (Batai polyclonal)). The slides were stained by incubating first with antisera followed by anti-human IgG or anti-mouse FITC conjugate. The slides were investigated with a fluorescence microscope.
If CPE was observed, an additional 10 µl of supernatant was used for EM studies. For EM studies, the samples were placed onto copper grids and were negatively stained with 2% KPTA tungstophosphoric acid (pH 6.0) and inspected with a transmission electron microscope (JEOL JEM-1400).

6.4 Molecular methods

6.4.1 PCR methods for screening (original publications II and III)

New virus isolates were initially confirmed with RT-PCR and, if positive, with sequencing. Total RNA was extracted using a QIAamp viral RNA mini kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed using Expand Reverse Transcriptase with the specific PCR primers Ink-S-FP 5′-agt agt gtg ctc cac ttg aat act and Ink-S-RP 5′-agt agt gtg ctc cac tga ata cat tt. The PCR was performed using *Taq* DNA polymerase with standard PCR protocols with the same primers.

Human serum samples of IgM-positive patients were screened with previously published PCR methods (Kuno et al. 1996, Fischer et al. 2013) that were modified to the Phusion flash PCR format. The PCR methods targeted regions of both the S and L segments.

6.4.2 Sequencing (original publication II)

All new virus isolates were grown in Cellmaster roller bottles (Greiner Bio-one) to gain a maximum amount of virus in the supernatant. The supernatant was ultracentrifuged for 20 hours at 24 000 rpm in an SW28 rotor, and the virus pellet was eluted in 200 µl TNE buffer (10 mM Tris-HCl, 1 mM EDTA, 150 mM NaCl). RNA was extracted as previously described using a Qiagen QIAamp viral RNA mini kit.

Sequencing primers for the complete S segment were Ink-S-FP 5′-agt agt gtg ctc cac ttg aat act and Ink-S-RP 5′-agt agt gtg ctc cac tga ata cat tt, and for the M segment, the primers were published previously (Campbell, Huang 1999). The beginning of the L segment was sequenced with the primers L-548 5′-tga aag tcg cac atg gtg at and L-3801 5′-tgc gca atc tcc tac aga tg. The last part of the L segment was sequenced using the primers in Appendix 2.

Sanger sequencing was used to complete sequencing of the S segment and the end of the L segment. Next-generation sequencing was performed with a 454 GS FLX genome sequencer using Titanium chemistry (Roche) as an outsourcing service at the Institute of Biotechnology, University of Helsinki and used for sequencing of the complete M gene and the beginning of the L segment.

6.4.3 Genomic and phylogenetic analysis (original publication II)

The Lasergene Seqman programme was used for raw data sequence analysis, and Mega5 (Molecular Evolutionary Genetics Analysis program), available online at http://megasoftware.net/ was used to obtain the similarity data.
Multiple alignments of the nucleotide sequences were calculated with the Clustal W programme, and phylogenetic trees were constructed by a Bayesian approach as implemented in the programme BEAST.

6.5 Viral protein expression and metabolic labelling (original publication I)

INKV RNA extraction and reverse transcription was performed as described above. The N, Gc and Gn protein coding regions were produced by PCR with specific primers containing suitable restriction sites. PCR products were digested, purified and ligated into the pAcYML1 vector, and constructs were transformed into *E. coli* DH5α. Bacteria containing the correct constructs were grown on a larger scale, and purified plasmids were transfected with baculovirus DNA and applied to *Spodoptera frugiperda* cells (Sf-9). Sf-9 cells expressing the correct proteins were harvested and used for the production of IFA slides or immunoprecipitation.

Native virus and expressed proteins were labelled with 50 µCi/ml L-[^{35}S] methionine and cysteine or 50 µCi/ml L-[^{35}S] methionine (Amersham Pharmacia Biotech) and immunoprecipitated with selected antibodies. Acute and convalescent serum samples and mouse hyperimmune ascites fluid (MHAF) were used. Briefly, infected cells were harvested after labelling, lysed and incubated with antibodies overnight. The proteins were allowed to bind with 20% G-Sepharose, subjected to sodium dodecyl sulfate-10% polyacrylamide gel electrophoresis, transferred to membranes, and detected on an X-ray film.

7 Results and discussion

7.1 Serodiagnostics

7.1.1 IgG and IgM IFA test for detecting California encephalitis virus infections (I)

A serological test was established to detect California serogroup virus antibodies from serum, blood and CFS samples. The test is used to detect IgG and IgM antibodies; the sensitivity, 87.5%, and the specificity, 90.7%, was determined by the gold standard neutralization assay. An IFA test is easy to perform in the laboratory compared to traditional NT and HI tests, and the results are obtained faster (within the same day); therefore, IFA using INKV-infected Vero E6 cells was established for routine laboratory use. The INKV IgG IFA assay was able to separate acute infection from pre-existing immunity with a distinct fluorescence pattern. Fluorescence of the acute phase serum appeared granular within the cell (figure 6b), while the fluorescence from previously immune serum was diffused throughout the entire infected cell (figure 6a). These results were also confirmed by protein-specific assays and immunoprecipitation assays. A similar IFA pattern was observed with other *Bunyaviridae*–family viruses, such as Puumala virus (Vapalahti et al. 1995). However, in practice, all suspected acute infections are confirmed with INKV IgM IFA.

The advantage of the IFA test is that all viral antigens are present and detected, compared with the NT and HI tests, which involve only glycoproteins. N protein is important in complement fixation. IFA is also suitable for specifically detecting IgM antibodies, and the

hands-on time is much shorter than that for conventional methods. The biggest disadvantage of the IFA test is the sensitivity of the assay; some of the infections may presumably not be due to Inkoo virus but another California serogroup virus.

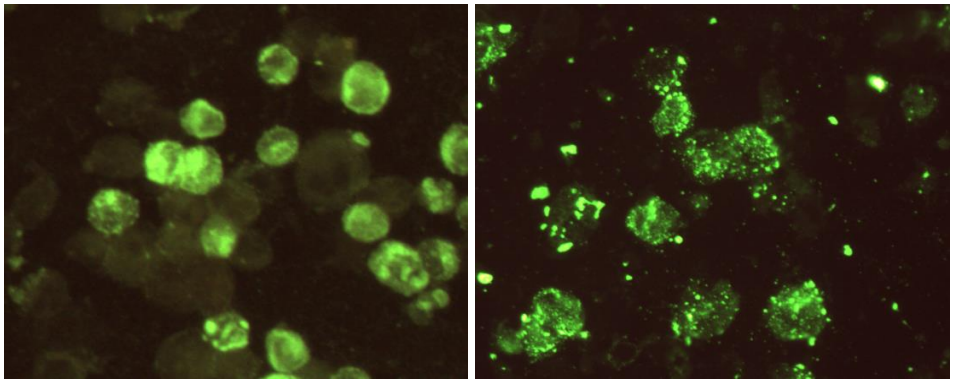

Figure 6. Fluorescence pattern in the INKV IFA test. a) previously immune sample in IgG IFA b) acute serum sample in IgG IFA.

In addition to the IFA assay, two different enzyme-linked immunosorbent assays (ELISAs) were established to detect INKV IgM antibodies. INKV-N protein, expressed by a baculovirus system, was used as antigen in μ-capture INKV IgM-ELISA, and concentrated whole INKV was used for direct ELISA (unpublished data). Both methods for INKV IgM antibody detection were sensitive, but the specificity did not reach the required standards. Both tests produced false positive results, i.e., cytomegalovirus and Epstein-Barr virus-positive samples were falsely identified as positive, and still, the cross-reaction ability within serogroups seemed lower than in IFA. Additionally, the repetitive production of the antigens proved difficult, and the stability in storage remained low (unpublished data).

7.1.2 Antibody response in INKV infection (I)

A baculovirus expression system and immunoprecipitation of the native virus was used in more detailed studies of the INKV antibody response, protein specificity and kinetics. Expression of the Inkoo virus N, Gc and Gn proteins was conducted to study the different fluorescence patterns observed in INKV IgG IFA between acute and previously immune serum samples. The successful production of INKV proteins was confirmed by IFA staining, where Sf-9 cells with baculovirus-expressed proteins were used as antigens. Slides were stained with acute, previously immune and MHAF antibodies (Original publication I, figure 4). The antibody response in the acute phase was primarily directed towards the N protein, while the response in previously immune samples was primarily directed towards glycoproteins (Original publication I, figure 4). Other studies have also shown that the antibodies against the N protein are mainly IgM class (Gonzalez-Scarano et al. 1982).

IFA studies with expressed proteins as antigens showed clearly how acute sera recognized only N protein and previously immune sera recognized Gc protein. The Gn protein expression level was presumably too low for the IFA format. Only high-titre mouse ascites could give a positive fluorescent signal. Gc protein fusion was feasible in transfected or infected Sf-9 cells. Sf-9 cells were fused during infection with recombinant baculovirus expressing the Gc protein, but a similar phenomenon was not observed with N and Gn protein constructs. Fusion was visible not only during cell culture (figure 7) but also in IFA slides (Original publication I, figure 4), although the cells were washed and suspended several times before fixing to the slides. A good production level of N proteins and the ability to recognize IgM antibodies give a good basis to use the protein as an antigen in IgM tests despite the fact the N protein cross-reacts between all California serogroup viruses. The Gc protein is needed for tests used in seroprevalence studies and to distinguish viruses in the serogroup from each other.

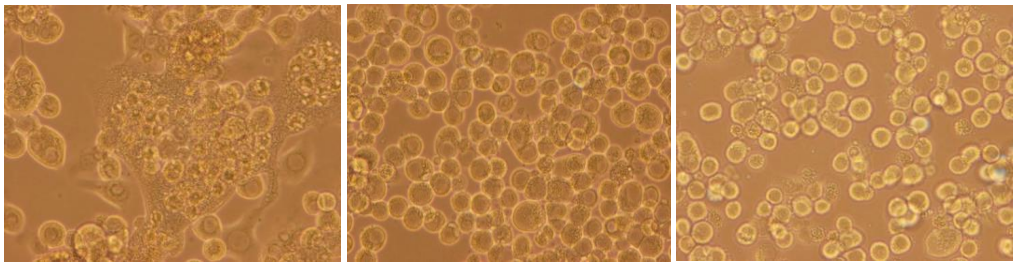

Figure 7. Sf-9 cells infected with baculovirus expressing a) INKV Gc protein b) INKV Gn protein and c) INKV N protein.

Similar to the results of IFAs using INKV protein-expressing cells, immunoprecipitation with acute and previously immune human serum samples demonstrated that the antibody response against the N protein is prevalent in acute infection and eventually the response develops against glycoproteins (original publication I, figure 5). IgM-positive samples were able to precipitate strongly with N protein (original publication I, figure 5a). However, when infection progressed, immunoprecipitation showed that the N protein response remained but weakened (original publication I, figure 5b). When evaluating the ratio between the N and Gc protein antibody response intensities, the value was smaller in the acute phase than in samples from previously infected individuals (original publication I, figure 5a and b).

The granular fluorescence pattern seems to occur as a result of N protein packing into the inclusion bodies (Tao et al. 1987). Furthermore, electron microscopy analysis has shown that the orthobunyavirus RNP has a highly ordered helical appearance, which might also be observed in the cytoplasm (Reguera et al. 2013). Because the specific response to N protein is strong throughout the acute infection, these structures are observed and the granular fluorescence pattern is observed in IFA. When a more extensive anti-Gc response is developed, the granular fluorescence is overpowered, and the cells fluorescence look more diffuse.

7.1.3 Seroprevalence of INKV infection in humans (I, IV)

Seroprevalence proved to be high (40-50%) in the studied populations, which indicates that California serogroup viruses frequently circulate in Finland and northern Sweden. The seroprevalence in Finland was 51% (males 59%, females 40%, figure 8) and in northern Sweden, it was 41% (male 47%, female 35%) (figure 9). The seroprevalence has increased in Finland from the previous studies; however, it was already then recognized to be high in northern Finland, with an up to 80% prevalence in the rural area of Lapland (Brummer-Korvenkontio 1973a). There are no previous data from Sweden of the California serogroup virus antibody prevalence in humans. The Ryazan region in Russia shares the same seroprevalence (41%) in humans as that observed in our studies. Yet this study from Russia did not distinguish the proportions of the total prevalence belonging to INKV and TAHV (Butenko et al. 1995). Similar to the INKV prevalence in Finland, TAHV infections seem to be frequent in Czechoslovakia, as 60% of the elderly have antibodies for TAHV (Hubalek 2008).

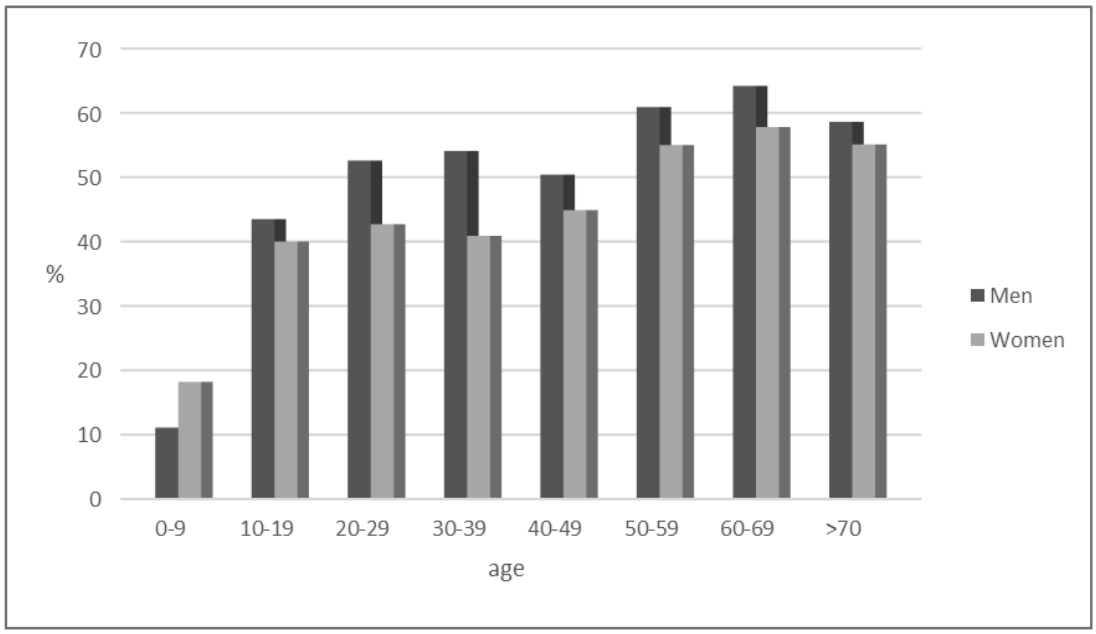

Figure 8. INKV IgG seroprevalence with respect to gender and age in Finland in 2001.

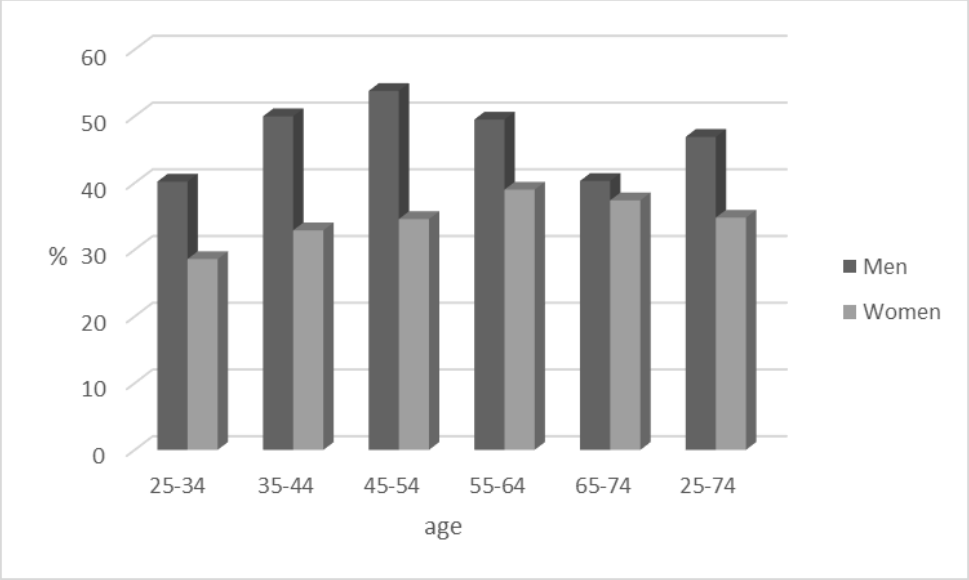

Figure 9. INKV IgG seroprevalence with respect to gender and age in northern Sweden in 2009.

The earlier INKV seroprevalence in southern and central Finland was 16%, in northern Finland was 44% and in most northern parts of Lapland was 69% (Brummer-Korvenkontio 1973a). In our study (figure 10), the seroprevalence differences between hospital districts are not as clear. The seroprevalence results show that infections with the California serogroup viruses have become more common in Finland. The seroprevalence in most districts is between 50-65%. Most likely, migration to the large cities from the countryside and the citizens' free time activities in summer cottages even out the differences in geographical seroprevalence. However, lacking knowledge on the natural reservoir of INKV and the abundance of the vectors, it is difficult to conclude the real reason for the current frequency. In addition, there are no real data regarding the CHATV seroprevalence in humans. Because there are also acute CHATV human infections (Putkuri et al. 2016), CHATV may have played a role in the increasing seroprevalence during the last decades. The INKV seroprevalence increased throughout all age groups and was somewhat higher in men. In Finland, children under 10 years had a relatively high seroprevalence, but the number of samples studied was low (69 out of 1292). The data indicate that INKV is encountered early in life and lifelong immunity is gained after the infection.

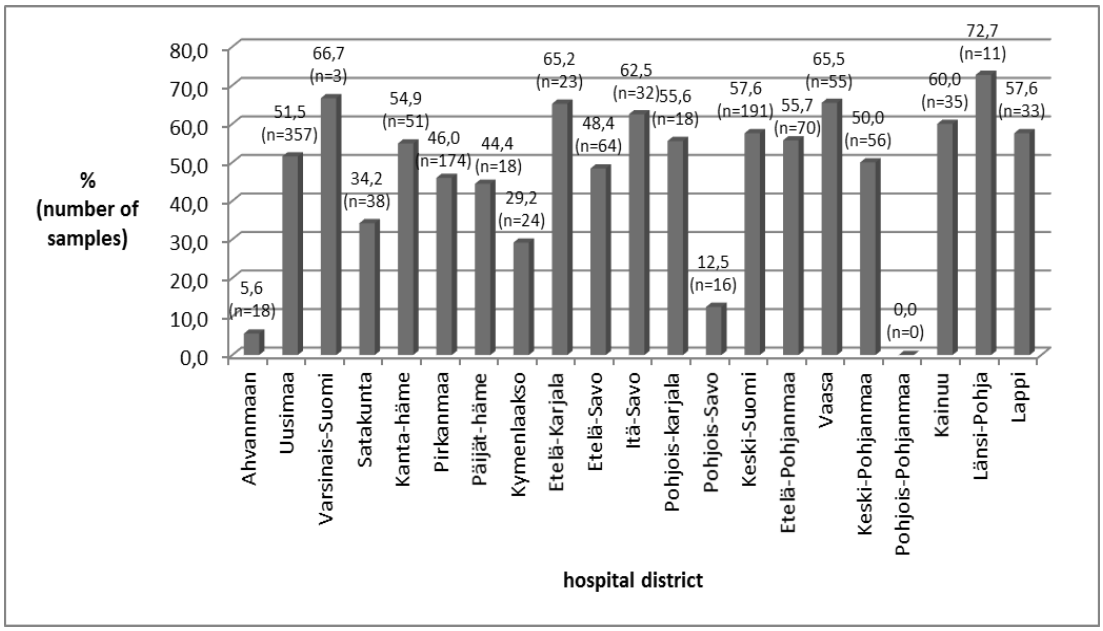

Figure 10. Geographical distribution (hospital districts) of INKV seroprevalence in Finland in 2001. The seroprevalence of each hospital district is shown as the percentage and total number of samples per hospital district.

7.2 New California serogroup viruses in Finland (II)

New California serogroup viruses were isolated in cell culture from mosquitoes collected in eastern Finland in 2007 and 2008 (original publication II, figure 1 and table 1). Altogether, five isolates were recovered in virus isolation attempts on mammalian cells; the viruses were named M07-1, M07-2-83, M07-2-84, S07-1 and ILO8-3. All except one of the positive isolates were collected in 2007; three of them originated from the Ilomantsi region in eastern Finland, and one was from the Sotkamo region. Only one isolate was obtained in 2008 from mosquitoes collected in the Ilomatsi region (original publication II, table 1). The first of these virus isolates was obtained from mosquitoes collected in Möhkö village of Ilomantsi, designating these as "Möhkö isolates". Since the collected mosquitoes were pooled in the process without prior entomological identification, little information is known about the host vectors. Suggestive results indicate that *Ochlerotatus* sp. and *Aedes* sp. mosquitoes are the most likely host vectors.

Morphologically, Möhkö isolates were similar with other viruses in family *Bunyaviridae* (figure 11). The virus particles were round and 80-110 nm in diameter. Sequencing revealed that the open reading frames (ORFs) of the S, M and L segments were 707 bp, 4328 bp and 6791 bp, respectively. The S segment was found to encode the putative ORF for the N and NSs proteins (235 and 79 amino acids); the M segment Gn, NSm and Gc proteins (299, 156, and 983 amino acids); and the L segment RNA polymerase coding region (2264 amino acids). All isolates were very similar, differing by less than 2% at the amino acid level from each other.

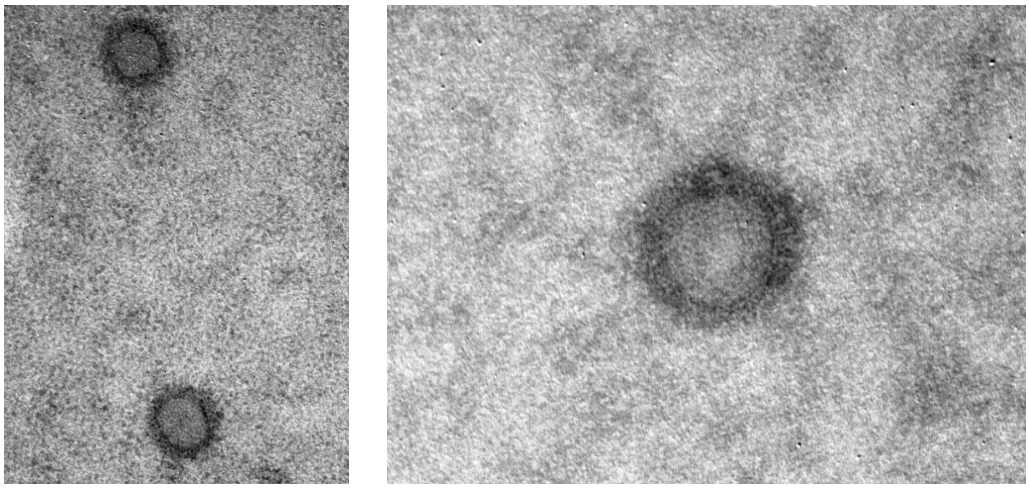

Figure 11. Electron microscopy image of Chatanga virus Möhkö isolate.

Segment similarity studies showed that the isolates had the highest similarity with CHATV (Table 3-5), which was originally found in mosquitoes collected in Taymarya, Russia in 1985 (Lavrent'ev et al. 2008). The N protein amino acid sequence similarity was less than 10% compared to other CEV isolates; therefore, as ICTV instructions indicate, the Möhkö isolates do not represent a new species in the genus. Instead, Möhkö isolates represent a new strain of CHATV. In Russia, a large quantity of viruses has been isolated from mosquitoes collected between 1985 and 1991; a total of 45 isolates of CHATV have been recovered thus far from mosquitoes collected throughout Russia (all sequences found in GenBank at NCBI). Finnish CHATV isolates were closely related to viruses isolated from the Komi region and areas close to Finland (Karelia, Archangel, and Murmansk). Yet, the amino acid identities with all CHATV isolates were at least 95,3%, 89,4% and 97,6% for N protein, glycoprotein and RNA polymerase, respectively (Table 3-5). When compared to the rest of the California serogroup viruses, Möhkö isolates proved to be most similar to SSHV and LACV.

Phylogenetic analysis based on the S segment and the partial M segment demonstrated that the CHATV isolates form 4 phylogroups instead of 3 in contrast to previous studies (figure 12) (Lavrent'ev et al. 2008, Poglazov, Shliapnikova & Prilipov 2011). The CHATV isolates close to Finland's eastern border formed their own phylogroup with the Möhkö isolates (phylogroup 1, Möhkö phylogroup)(original publication II, figure 3). In addition, phylogroup 3 could be divided into two subgroups, a and b; however, this was based on only S and partial M segment analysis since the isolates from group 3a were not fully sequenced and comparison analysis with these viruses could not be performed with complete M and L segments (original publication II, figure 3).

Table 3. L segment (longer than the ORF of RNA polymerase) amino acid similarity (%) within the California serogroup, including all Möhkö isolates from Finland. The isolates named LEIV-15854, LEIV- 17756, LEIV-18733 and LEIV-22712 are different Chatanga virus isolates from Russia, and ILO8-3, M07-1, M07-2-83, M07-2-84 and S07-2 are different Möhkö isolates from Finland.

	INKV	JCV	TAHV	LEIV-15854	LEIV-17756	LEIV-18733	LEIV-22712	SSHV	LACV	ILO8-3	M07-1	M07-2-83	M07-2-84	S07-2
INKV	ID	96,7	82,4	83,1	83,0	83,0	83,2	83,0	82,9	83,1	82,9	82,9	83,0	82,9
JCV	96,7	ID	83,1	83,3	83,2	83,3	83,2	83,3	83,4	83,2	83,1	83,1	83,2	83,1
TAHV	82,4	83,1	ID	89,3	89,6	89,7	89,1	89,9	89,1	89,3	89,2	89,2	89,3	89,2
LEIV-15854	83,1	83,3	89,3	ID	97,9	97,9	97,8	95,9	93,9	99,6	99,3	99,3	99,4	99,3
LEIV-17756	83,0	83,2	89,6	97,9	ID	99,5	98,1	96,0	93,6	97,8	97,7	97,7	97,7	97,7
LEIV-18733	83,0	83,3	89,7	97,9	99,5	ID	98,0	96,1	93,7	97,8	97,7	97,7	97,7	97,7
LEIV-22712	83,2	83,2	89,1	97,8	98,1	98,0	ID	95,4	93,6	97,7	97,7	97,6	97,7	97,6
SSHV	83,0	83,3	89,9	95,9	96,0	96,1	95,4	ID	94,6	96,0	95,9	95,8	95,9	95,8
LACV	82,9	83,4	89,1	93,9	93,6	93,7	93,6	94,6	ID	93,9	93,7	93,7	93,8	93,7
ILO8-3	83,1	83,2	89,3	99,6	97,8	97,8	97,7	96,0	93,9	ID	99,5	99,5	99,6	99,5
M07-1	82,9	83,1	89,2	99,3	97,7	97,7	97,7	95,9	93,7	99,5	ID	99,7	99,8	99,8
M07-2-83	82,9	83,1	89,2	99,3	97,7	97,7	97,6	95,8	93,7	99,5	99,7	ID	99,8	99,7
M07-2-84	83,0	83,2	89,3	99,4	97,7	97,7	97,7	95,9	93,8	99,6	99,8	99,8	ID	99,8
S07-2	82,9	83,1	89,2	99,3	97,7	97,7	97,6	95,8	93,7	99,5	99,8	99,7	99,8	ID

The phylogroups seemed to have some geographic association (original publication II, figure 1). Only the Tyumen region in Russia had several isolates from different phylogroups circulating in the same area. Phylogroup 1 (Möhkö phylogroup) circulates in eastern Finland and western parts of Russia, phylogroup 2 in eastern Russia and northern parts of east Siberia (north Yakutia), phylogroup 3a in southern Siberia (Altai), 3b in southern parts of east Siberia (south Yakutia) and phylogroup 4 in western Siberia (Tyumen) (Lavrent'ev et al. 2008). Phylogenetic analysis confirmed that CHATV strains are more closely related to the California serogroup viruses circulating mostly in USA and previously known to belong to California encephalitis virus complex. Additionally, INKV is more distinct from CHATV than the third European representative of the California serogroup, TAHV. Interestingly, the closest of both viruses circulating in Finland are found in the USA. INKV is almost identical with JCV, and CHATV shares 86-96% of the genome similarity with SSHV (Putkuri et al. 2014, Vapalahti et al. 1996). It is possible that CHATV originates from SSHV, the distribution of has been thought to reach Russia based on the serological data. However, the genomic similarity of SSHV to CHATV might have caused the cross-reaction and the false result of SSHV existence in Russia.

Table 4. M segment polyprotein aa similarities (%) within the CEV group, including all Möhkö isolates from Finland. The isolates named LEIV-15854, LEIV- 17756, LEIV-18733 and LEIV-22712 are different Chatanga virus isolates from Russia, and ILO8-3, M07-1, M07-2-83, M07-2-84 and S07-2 are Möhkö isolates from Finland.

	TAHV	LEIV-15854	LEIV-22712	LEIV-17756	LEIV-18733	SSHV	LACV	CEV	SAV	INKV	JCV	SROV	MELV	SDNV	KEYV	M07-1	M07-2 -84	M07-2-83	S07-2	IL08-3
TAHV	ID	77,0	77,0	77,3	76,4	76,5	74,8	76,5	71,7	67,6	68,1	68,4	69,4	68,8	68,8	77,0	77,1	77,1	77,1	76,9
LEIV-15854	77,0	ID	89,6	91,2	91,4	86,3	82,6	76,8	72,6	67,9	67,9	68,5	69,0	69,1	67,9	97,6	97,8	97,8	97,8	98,4
LEIV-22712	77,0	89,6	ID	92,5	90,3	86,8	83,4	77,5	71,7	68,2	68,9	68,7	69,4	68,6	68,0	89,4	89,5	89,5	89,5	89,4
LEIV-17756	77,3	91,2	92,5	ID	91,8	87,8	83,9	77,7	72,5	68,6	68,0	68,4	69,2	68,2	67,9	91,9	92,0	92,0	92,0	91,6
LEIV-18733	76,4	91,4	90,3	91,8	ID	86,7	83,7	77,7	72,5	68,0	68,5	68,9	68,9	68,8	67,9	91,2	91,4	91,4	91,4	91,2
SSHV	76,5	86,3	86,8	87,8	86,7	ID	84,8	77,5	71,7	69,2	68,7	69,0	69,9	68,3	67,7	86,9	86,9	86,9	86,9	86,5
LACV	74,8	82,6	83,4	83,9	83,7	84,8	ID	76,7	71,4	67,2	68,1	68,1	69,1	67,6	67,8	82,2	82,3	82,3	82,3	82,0
CEV	76,5	76,8	77,5	77,7	77,7	77,5	76,7	ID	72,9	66,8	67,1	67,6	69,4	68,0	67,1	76,5	76,6	76,6	76,6	76,8
SAV	71,7	72,6	71,7	72,5	72,5	71,7	71,4	72,9	ID	65,4	66,5	66,2	67,1	66,6	65,6	72,1	72,2	72,2	72,2	72,2
INKV	67,6	67,9	68,2	68,6	68,0	69,2	67,2	66,8	65,4	ID	89,2	87,3	73,4	72,8	69,9	68,3	68,4	68,4	68,4	68,3
JCV	68,1	67,9	68,9	68,0	68,5	68,7	68,1	67,1	66,5	89,2	ID	89,6	74,6	74,4	70,8	68,2	68,2	68,2	68,2	67,9
SROV	68,4	68,5	68,7	68,4	68,9	69,0	68,1	67,6	66,2	87,3	89,6	ID	74,9	75,5	71,2	68,5	68,5	68,5	68,5	68,3
MELV	69,4	69,0	69,4	69,2	68,9	69,9	69,1	69,4	67,1	73,4	74,6	74,9	ID	79,9	73,8	68,7	68,8	68,8	68,8	69,2
SDNV	68,8	69,1	68,6	68,2	68,8	68,3	67,6	68,0	66,6	72,8	74,4	75,5	79,9	ID	74,6	68,6	68,6	68,6	68,6	68,6
KEYV	68,8	67,9	68,0	67,9	67,9	67,7	67,8	67,1	65,6	69,9	70,8	71,2	73,8	74,6	ID	68,1	68,2	68,2	68,2	67,9
M07-1	77,0	97,6	89,4	91,9	91,2	86,9	82,2	76,5	72,1	68,3	68,2	68,5	68,7	68,6	68,1	ID	99,8	99,8	99,8	98,2
M07-2-84	77,1	97,8	89,5	92,0	91,4	86,9	82,3	76,6	72,2	68,4	68,2	68,5	68,8	68,6	68,2	99,8	ID	100,0	100,0	98,3
M07-2-83	77,1	97,8	89,5	92,0	91,4	86,9	82,3	76,6	72,2	68,4	68,2	68,5	68,8	68,6	68,2	99,8	100,0	ID	100,0	98,3
S07-2	77,1	97,8	89,5	92,0	91,4	86,9	82,3	76,6	72,2	68,4	68,2	68,5	68,8	68,6	68,2	99,8	100,0	100,0	ID	98,3
IL08-3	76,9	98,4	89,4	91,6	91,2	86,5	82,0	76,8	72,2	68,3	67,9	68,3	69,2	68,6	67,9	98,2	98,3	98,3	98,3	ID

Table 5. S segment N protein aa similarities (%) within the CEV group, including all Möhkö isolates from Finland. The isolates named LEIV-15854, LEIV- 17756, LEIV-18733 and LEIV-22712 are different Chatanga virus isolates from Russia, and ILO8-3, M07-1, M07-2-83, M07-2-84 and S07-2 are Möhkö isolates from Finland.

	LACV	SANV	SDNV	SROV	SSHV	LEIV-17756	LEIV-15854	LEIV-18733	LEIV-22712	KEYV	MELV	TVTV	JCV	TAHV	CEV	INKV	ILO8-3	M07-1	S07-2	M07-2-83	M07-2-84
LACV	ID	86,3	80,4	82,9	90,6	89,7	90,6	90,6	90,6	80,4	82,9	73,1	82,9	86,3	85,9	83,4	90,2	90,6	89,7	90,2	90,6
SANV	86,3	ID	82,5	85,5	88,5	88,9	88,5	88,9	89,3	85,1	84,6	73,1	83,8	89,3	88,9	84,2	88,9	88,5	87,6	88,0	88,0
SDNV	80,4	82,5	ID	91,4	80,0	81,7	81,2	81,7	82,1	88,5	87,6	76,1	91,0	82,5	83,8	91,4	80,8	81,2	80,4	80,8	80,8
SROV	82,9	85,5	91,4	ID	82,5	84,6	85,1	85,1	85,1	88,5	88,9	75,7	97,8	84,2	85,9	98,2	84,6	85,1	84,2	84,6	84,6
SSHV	90,6	88,5	80,0	82,5	ID	93,6	93,1	93,6	93,1	80,8	82,1	73,1	81,7	88,5	88,9	82,1	92,7	93,1	92,3	92,7	92,7
LEIV-17756	89,7	88,9	81,7	84,6	93,6	ID	97,8	96,5	98,7	83,4	83,8	74,0	84,2	90,2	89,3	84,6	97,4	97,8	97,0	97,4	97,4
LEIV-15854	90,6	88,5	81,2	85,1	93,1	97,8	ID	96,1	98,2	83,8	83,8	73,6	84,6	89,7	88,9	85,1	99,5	100,0	99,1	99,5	99,5
LEIV-18733	90,6	88,9	81,7	85,1	93,6	96,5	96,1	ID	97,8	82,5	83,8	73,1	84,2	88,5	88,5	84,6	95,7	96,1	95,3	95,7	95,7
LEIV-22712	90,6	89,3	82,1	85,1	93,1	98,7	98,2	97,8	ID	83,8	84,2	73,6	84,6	90,6	89,7	85,1	97,8	98,2	97,4	97,8	97,8
KEYV	80,4	85,1	88,5	88,5	80,8	83,4	83,8	82,5	83,8	ID	87,6	74,8	87,6	83,4	84,6	88,0	84,2	83,8	82,9	83,4	83,4
MELV	82,9	84,6	87,6	88,9	82,1	83,8	83,8	83,8	84,2	87,6	ID	75,3	88,0	84,6	82,9	88,0	83,4	83,8	82,9	83,4	83,4
TVTV	73,1	73,1	76,1	75,7	73,1	74,0	73,6	73,1	73,6	74,8	75,3	ID	75,3	74,0	74,0	75,7	73,6	73,6	72,7	73,1	73,1
JCV	82,9	83,8	91,0	97,8	81,7	84,2	84,6	84,2	84,6	87,6	88,0	75,3	ID	84,6	85,1	99,5	84,2	84,6	83,8	84,2	84,2
TAHV	86,3	89,3	82,5	84,2	88,5	90,2	89,7	88,5	90,6	83,4	84,6	74,0	84,6	ID	90,2	84,6	89,3	89,7	88,9	89,3	89,3
CEV	85,9	88,9	83,8	85,9	88,9	89,3	88,9	88,5	89,7	84,6	82,9	74,0	85,1	90,2	ID	85,5	88,5	88,9	88,0	88,5	88,5
INKV	83,4	84,2	91,4	98,2	82,1	84,6	85,1	84,6	85,1	88,0	88,0	75,7	99,5	84,6	85,5	ID	84,6	85,1	84,2	84,6	84,6
ILO8-3	90,2	88,9	80,8	84,6	92,7	97,4	99,5	95,7	97,8	84,2	83,4	73,6	84,2	89,3	88,5	84,6	ID	99,5	98,7	99,1	99,1
M07-1	90,6	88,5	81,2	85,1	93,1	97,8	100,0	96,1	98,2	83,8	83,8	73,6	84,6	89,7	88,9	85,1	99,5	ID	99,1	99,5	99,5
S07-2	89,7	87,6	80,4	84,2	92,3	97,0	99,1	95,3	97,4	82,9	82,9	72,7	83,8	88,9	88,0	84,2	98,7	99,1	ID	98,7	98,7
M07-2-83	90,2	88,0	80,8	84,6	92,7	97,4	99,5	95,7	97,8	83,4	83,4	73,1	84,2	89,3	88,5	84,6	99,1	99,5	98,7	ID	99,1
M07-2-84	90,6	88,0	80,8	84,6	92,7	97,4	99,5	95,7	97,8	83,4	83,4	73,1	84,2	89,3	88,5	84,6	99,1	99,5	98,7	99,1	ID

The principal host for CHATV is not known. The SSHV host *Lepus americanus* (Burgdorfer, Newhouse & Thomas 1961) is widespread in North America, whereas *Lepus timidus* (mountain hare) is found from eastern Siberia to Fennoscandia. The geographic range of *Lepus timidus* seems to resemble CHATV distribution. Molecular clock analysis shows that these viruses seem to have had intercontinental spread, and according to M segment phylogeny, the direction might have been from North America (with more ancestral nodes) to northeast Russia (unpublished data). It could be possible that the host switch of SSHV from *Lepus americanus* to *Lepus timidus* has created CHATV, which has evolved later. However, the S and L phylogenies show the American and European snowshoe hare-like viruses as sister taxa without the ability to confirm the direction of the intercontinental spread. The vertebrate host of INKV is still unclear, but it is most likely a bigger mammal, such as moose or deer. Because INKV is closely related to Jamestown canyon virus, the vertebrate host of which is known to be white-tailed deer, it is tempting to speculate whether the ancestor of INKV (aka JCV) was introduced to Finland when the Minnesota Finns Deer Committee gave white-tailed deer as a gift to Finland in 1934 and 1948 and that the virus has then adapted to the local deer/moose population and mutated into INKV.

Möhkö prototype isolate (M07-1) was compared using cross-neutralization and haemagglutination inhibition analysis to INKV, SSHV and TAHV viruses, and INKV and TAHV antibodies antibodies were used (original publication II, Table 3). The results confirm that Möhkö isolates belong to the California serogroup (could be neutralized by other virus antibodies), but the titre difference between the viruses remained small. Clearer results could be achieved if specific antibodies against each tested virus were available. The infectivity of prototype Möhkö isolate(s) was tested with different cell types, and the result did not differ from those obtained with INKV.

With regard to the vector species of Möhkö isolates, the retrospective genetic identification of the mosquito species suggests that the vector(s) most probably belong to *Ochlerotatus* (former *Aedes* species) or *Aedes* species. Most of the cox I clone sequences obtained from MÖHKV-positive pools resembled *Ochlerotatus cantans*, *Ochlerotatus annulipes* or *Aedes cinereus*, which therefore could be the possible host vector. However, *Ochlerotatus punctor* and *Ochlerotatus communis*, which are the primary vectors for INKV, cannot be excluded either. The results are approximate because the studied mosquitoes were pooled. The mosquito population density is the largest during the midsummer, and yet it seems that the MÖHKV prevalence in mosquitoes seems to be highest at the end of the autumn.

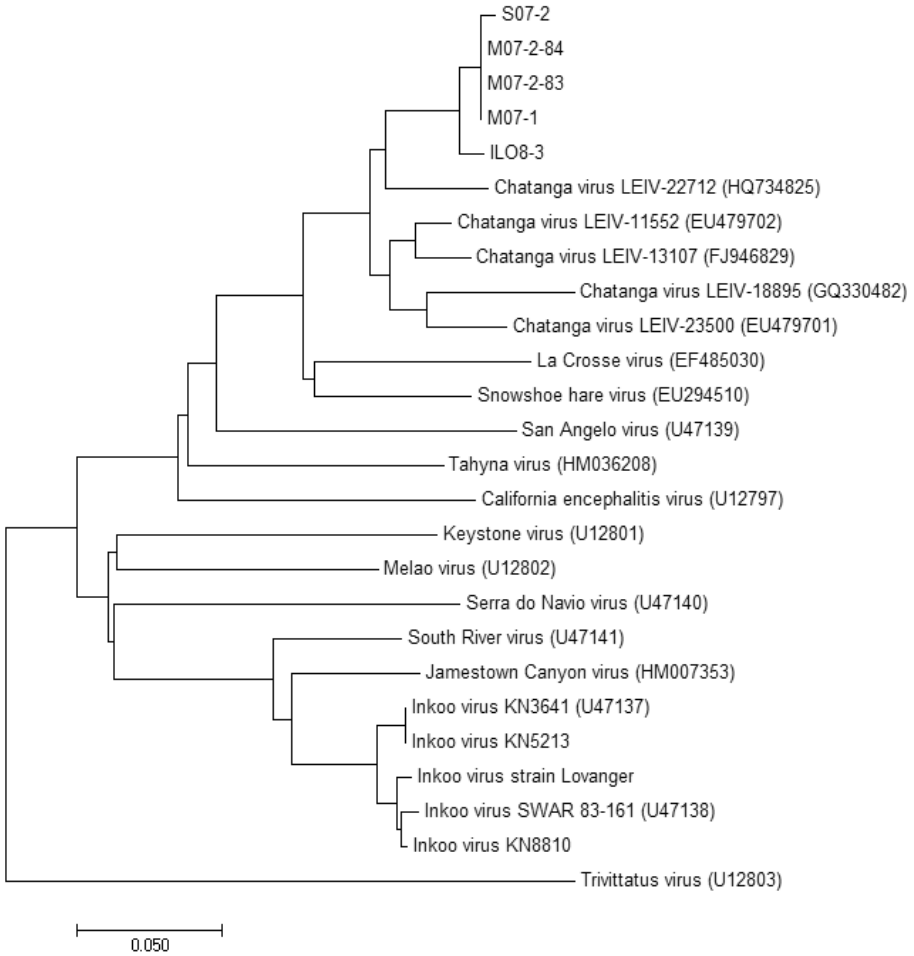

Figure 12. Phylogenetic tree of a partial S segment (length 596 nucleotides) of California serogroup viruses (unpublished results). The phylogenetic tree was constructed using the neighbour-joining method.

California encephalitis group viruses are capable of forming reassortments between virus isolates. European CEV have appear to circulate in distinct areas. TAHV is circulating in central Europe, Norway and European Russia; INKV circulates in Scandinavia and western Russia; and SSHV circulates in northern Russia. Thus, European Russia and Norway have been the only places in Europe where more than one of these viruses have been reported to circulate in the same area. Here, we demonstrate that Finland is also an area where two California encephalitis virus isolates circulate – or at least have been circulating.

7.3 INKV and CHATV infections in humans (III)

7.3.1 Frequency of acute infections in humans

Serum samples from selected patient groups were screened for INKV IgM antibodies in order to study the frequency of acute Inkoo virus infection. The samples were initially screened for the causative agents of CNS infection (HSV1, HSV2, HHV6, VZV, *Mycoplasma pneumoniae*), Puumala virus or specifically for INKV during the mosquito season. Study material had been collected since 2001, and 7961 serum and 832 CSF samples were tested. In total, 15 IgM-positive patients were identified by screening of serum samples, five of those with suspected Puumala virus infection and nine with neurological symptoms. One patient sample was sent to the laboratory specifically for INKV antibody testing, and it tested positive. All CSF samples screened were negative. Data from two acute Inkoo virus infections identified earlier in 1976 and 1980 were also included. The case reports of these patients had been published earlier in a domestic medical journal (Putkuri et al. 2004).

The serological results were confirmed with a neutralization test. Interestingly, among the 15 IgM-positive samples both INKV and CHATV positivity was confirmed by PRNT (Table 6). INKV was more prevalent (14/17); however, at least 2/17 patients were confirmed as CHATV-positive; the titre was 4 times higher than with the other viruses. This is the first time that CHATV has been shown to infect humans. The titre difference in one sample did not reach the 4-fold level required to meet diagnostic criteria, but presumably, this patient has had CHATV infection rather than INKV since no travel history was found in the patient files.

The IgG and IgM titres varied considerably between samples. All but two individuals already had IgG antibodies at the time they were identified as IgM-positive. The neutralisation titre was higher than 20 000 in one of the CHATV-positive samples (Table 6).

The annual number of samples, age range and gender ratio of the IgM-positive cases is shown in original publication III in Table 1. The frequency of acute INKV and CHATV infections in the studied patient groups in Finland varied from 0 to 5 cases per year. The percentage of IgM-positive serum samples among patients with CNS symptoms, suspected Puumala virus or INKV infection were 0,21% (9/4214), 0,14% (5/3574) and 0,58% (1/173), respectively. In comparison diagnosed LACV infections in the USA can reach up to 200 cases annually; 50-200 cases have been diagnosed annually between 1964 and 2013 (data published by CDC), while 5-20 clinical TAHV infections are diagnosed annually (Hubalek 2008, Sluka 1969). Other CEV isolates are less frequently diagnosed and associated with clinical disease (SSHV and JCV) in North America. In our study, the number of acute INKV and CHATV infections found annually in Finland was comparable to that of LACV infections in the USA.

Table 6. Serological analysis of all IgM-positive patient samples. The table shows the IgG and IgM IFA results and the plaque reduction neutralization test results for INKV, TAHV and CHATV.

	IgG IFA titre	IgM IFA titre	PRNT50 titre		
			INKV	CHATV	TAHV
1	160	160	320	40	40
3	120	40	320	40	40
5 †	160	+/ND	ND	ND	ND
6 †	320	+/ ND	ND	ND	ND
7	80	>320	>640	40	80
8	320	40	320	<20	<20
9	80	80	160	40	<40
10	40	20	160	20	40
11	320	>320	320	40	80
12	<20	40	320	20	40
13	160	120	160	40	40
14	<20	120	>640	<20	20
15	320	120	640	40	40
17	80	30	320	<20	<20
16	960	40	1280	**5120**	1280
2	80	40	320	**640**	40
4	>640	>320	320	**20480**	5120

The majority of the IgM-positive cases (88%) were detected in August or later. This indicates that INKV and CHATV are spread mainly by horizontal transmission; the number of infected mosquitoes increases during the summer months, when more mosquitoes come in contact with the amplifying hosts. However, there is some evidence of transovarial transmission since reports from Russia show another peak of acute cases in May, which could mean the first generation of mosquitoes hatched from eggs are carrying the virus (Butenko et al. 1995). LACV is transmitted by both horizontal and vertical transmission, and acute human cases are found in all summer months (Haddow, Odoi 2009).

La Crosse virus infection is a well-known arboviral neuroinvasive disease in children in the USA, and the majority of the reported acute SSHV infections have also been diagnosed in children under 10 years old (Grimstad et al. 1982, Fauvel et al. 1980, Meier-Stephenson et al. 2007, Embil, Camfield & Artsob 1985, Embil et al. 1982, Artsob 1985) or in young adults (Artsob et al. 1981). A recent study showed that JCV is a more important pathogen for adults based on the number of acute cases; 84% of the acute cases found were in individuals over 20 years old (Pastula et al. 2015). In our study, acute Inkoo virus infections were found in all age groups, and the age range of the patients was from 7 to 81 years. IgM antibodies to INKV were most commonly observed in patients under 19

Table 7. Confirmed INKV and CHATV infections found in samples collected between 2001 and 2013 shown by age group, sex of the patients and geographical distribution. Modified from Table 2 from original publication III.

Variables		INKV infection	CHATV infection	Positivity rate
Age range	**Total no. of patients**			
0-9	670	1	-	0,15
10-19	717	2	-	0,28
20-29	905	-	-	-
30-39	1062	1	-	0,10
40-49	1180	2	1	0,25
50-59	1377	5	1	0,44
60-69	1093	1	-	0,09
>70	957	-	1	0,11
Sex	**N**			
Female	3786	8	-	0,21
Male	4129	4	3	0,17
Region	**N**			
Lappi	118	-	-	-
Länsi-Pohja	19	-	-	-
Kainuu	162	-	-	-
Pohjois-Pohjanmaa	29	-	-	-
Keski-Pohjanmaa	131	-	-	-
Etelä-Pohjanmaa	75	-	-	-
Pohjois-Karjala	338	-	1	0,30
Pohjois-Savo	88	-	-	-
Itä-Savo	122	2	-	1,63
Etelä-Karjala	117	-	-	-
Etelä-Savo	146	-	-	-
Keski-Suomi	722	1	-	0,14
Vaasa	152	-	-	-
Satakunta	41	-	-	-
Pirkanmaa	331	-	1	0,30
Kanta-Häme	309	-	-	-
Päijät-Häme	256	1	-	0,39
Kymenlaakso	419	-	-	-
Uusimaa	4311	8	1	0,21
Varsinais-Suomi	5	-	-	-
Ahvenanmaa	70	-	-	-

-; no cases, INKV=Inkoo virus, CHATV=Chatanga virus.

years (20%) or those between 50 and 59 (33%) (Table 7). Previous studies have shown that 80-90% of La Crosse virus infections occur in children under 15 years, and approximately 65% occur in males (Haddow, Odoi 2009, Kappus et al. 1983). Only 20% of the acute INKV cases were identified in children under 16 years, but the disease seemed more severe. The INKV infection risk did not seem generally higher in males; the gender distribution was even (8 females, 6 males). Yet 3 out 4 children with INKV infection were males. In contrast, the CHATV cases were all male patients over 40 years old (Table 7 and original publication III, table 4).

Geographically, most of the confirmed cases were from southern Finland (9/15) (Table 7 and original publication III, figure 1). However, in our study 54% of samples were collected in health care settings in southern Finland. The highest cluster of cases was in the Helsinki region, but the positivity rate in this region was below avarage (Table 7). Only 4% of the samples were from northern parts of Finland (Lappi), where at least 60% of the population has previously been shown to be infected with INKV (Table 7). If we are able to identify acute CEV cases from the lowest seroprevalence areas annually, it is reasonable to assume that in northern Finland, acute cases could more prevalent. Interestingly, in eastern Finland the positivity rate was 7 times higher in certain areas (Itä-Savo) compared to southern Finland. The low IgM prevalence in selected patient groups could indicate that INKV and CHATV infections are mostly mild infections and rarely require visits to health care facilities. However, the true incidence rate remains to be studied when more samples are investigated from other regions of Finland. In comparison, the LACV mean incidence risk is 30,2 per 100 000 persons for children 15 years and younger (Haddow, Odoi 2009, Kappus et al. 1983).

7.3.2 Clinical characteristics of the acute disease in humans

A clinical history of 17 patients with acute Inkoo virus or Chatanga virus infection was reviewed from medical notes. Most of the patients with acute INKV and CHATV infection were not hospitalized. Most of the patients (10/17) were treated because of some other diagnosed infectious diseases or underlying disease, but the role of INKV or CHATV infection in these patients is unclear. Patients who presented symptoms that were likely due to acute INKV or CHATV infections (7/17) did not have any other underlying disease or laboratory findings implying another infection (Table 3 from original publication III). One patient out of the 7 presumed acute INKV and CHATV infections visited an outpatient clinic presenting fever and flu-like illness and recovered fully after 3 days. The rest of the patients (6/7) were hospitalized, and the patient histories are presented in table 8.

Table 8. Patient histories of the hospitalized INKV and CHATV IgM-positive patients. Modified from table 4 from original publication III.

Patient history	1 INKV	2 INKV	3 INKV	4 INKV	1 CHATV	2 CHATV
	8 years old Day 1: Fever, 38°C, flu-like symptoms Day 3: Disoriented Day 6: Hospitalized, abnormal EEG CAL IgM+ Day 7: Psychotic, compulsory thoughts, discharged Day 10: EEG: still same abnormalities 3 months later: EEG almost normal	17 years old Day 1: Fever, 39.5°C, headache, nuchal rigidity. Sore throat before fever => Hospitalized Day 2: Nuchal rigidity and headache deteriorating. Slowness, yet oriented Day 3: Discharged CAL IgM+ Day 5: Headache again => hospitalized. Still no abnormal laboratory findings. Day 6: Discharged again	12 years old Day 1: Vomiting => Hospitalized Day 2: Stomach pain, diarrhoea, seizures Day 3: Fever, 38.3°C, drowsiness, convulsions Day 4: More seizures, small changes in EEG Day 5: CAL IgM+ Day 8: Discharged	16 years old Day 1: Fever, 37.9°C, sore throat Day 3: CAL IgM+ Day 4: Nausea and vomiting Day 5: Fever, 39°C, headache, nuchal rigidity => hospitalized Day 10: Recovered totally, discharged	82 years old Day 1: Vomiting continues for 3 days Day 4: Fever => Hospitalized, disoriented during night Day 7: Frontal headache. Head CT normal, abdominal ultrasound normal Day 12: Discharged, CAL IgM+	54 years old Back injury two weeks earlier Day 1: Fever 39°C, back pain Day 7: Hospitalized, high fever, back pain almost resolved Day 7-22: Temporal pain, trembling of hands, fluctuating fever Day 17: CAL IgM+ Day 23: Discharged

The patient histories from these six patients indicated they presented to the hospital with febrile or flu-like symptoms (table 8). In our study, the most prominent symptom was fever (6/6), often together with sore throat. Other symptoms included headache (5/6), nausea and/or vomiting (3/6), disorientation (2/6), nuchal rigidity (2/6) and drowsiness (1/6). Seizures were present in one patient, whereas LACV infection is associated more often with seizures (46%; (McJunkin et al. 2001)). These symptoms are in line with the clinical characteristics of other CEV infections (LACV, JCV, TAHV) in humans (Pastula et al. 2015, Sluka 1969, McJunkin et al. 2001).

Adults suffered febrile illness, in contrast to children, who had CNS symptoms. Hospitalization was required in 6 out of 7 cases because of the acute INKV or CHATV infection. All hospitalized patients recovered fully. Most importantly, all children under 16 years with acute INKV infection were hospitalized because of more severe disease. This resembles the features of neuroinvasive LACV infection (Haddow, Odoi 2009). Yet, all children were discharged within one week from admission, similar to the duration of illness from LACV infection, which typically lasts from 3 to 9 days (McJunkin et al. 2001). Adult patients with acute INKV infections in our study visited outpatient clinics only and did not require hospitalization.

In contrast to INKV infection, acute CHATV infection was found only in adults. In addition, 2 out of 3 patients were hospitalized, and the hospital stay took more than a week before the patients recovered fully. CHATV infection somewhat resamples JCV infection, which also infects more adults (Pastula et al. 2015). However, JCV has been shown to cause meningoencephalitis in 65% of cases, whereas no CNS disease was diagnosed among the CHATV patients in our study. The most recently published JCV case was a 62-year-old male (Rogstad et al. 2015). The clinical picture of this patient was considered viral sepsis rather than flu-like illness or neuroinvasive disease, differing from the previous findings. Taking this into consideration, our patient material was highly selective and concentrated on finding CNS disease. The frequency of acute infections was low, and it raised the question of whether these viruses can be associated with symptoms other than those experienced by the patients included in our material.

In our study, co-infections with other pathogens were quite usual (6/17). Few patients were coinfected with herpes virus (cold sores), which has also been described in TAHV infections (Sluka 1969). In addition, acute Puumala virus infection, an *Escherichia coli* urosepsis and impetigo contagiosa were diagnosed. Studies in Russia indicated that mixed infections generated more severe INKV infection (Demikhov, Chaitsev 1995). Other infectious diseases and trauma seem to predispose patients to INKV infection. This may reflect the inability of INKV to infect healthy adults, which would explain why the virus is associated with more severe disease in children. Weakening of the immune system of adults might be necessary for INKV to cause clinical disease.

None of our IgM-positive samples were PCR-positive; therefore, virus isolation from humans or isolated INKV or CHATV RNA from human samples remains unsuccessful. This would be important since LACV strains isolated from fatal cases have shown that the human isolates form one separate linage together with mosquito isolates obtained from the same area (Huang et al. 1997, Lambert et al. 2015). This indicates that a restricted range of LACV genotypes is associated with severe clinical cases. This could also be the case with INKV and CHATV; only certain strains with a low frequency in mosquitoes might be responsible for the acute infections that require medical attention.

8 Concluding remarks and future prospects

The Californian serogroup includes important human pathogens and is widely distributed. This serogroup is more well-recognized in the USA, where LACV is the most important arbovirus causing CNS disease in children. Elsewhere, the California serogroup has been largely forgotten, yet there is an abundance of serological data indicating that these viruses commonly infect humans. In our studies, both in Finland and Sweden, the seroprevalence in the studied populations reached 50%. Human contact with these viruses occurs early in life.

INKV and CHATV are the OBV representatives found in Finland. CHATV was isolated from East Finland, yet the true distribution area here requires more research. We established a

serological IgG and IgM IFA test to detect these infections and to simplify the current diagnostic process. The IgG IFA test could be used solely since it can distinguish serum samples from those with acute infections and those with pre-existing immunity using distinct fluorescent signals. Serological studies showed that California serogroup virus infections are common since the seroprevalence in the studied populations remains high. The INKV seroprevalence among humans was shown to already be high when the virus was isolated in the 1970s. In our study, there was not as large a geographical difference in seroprevalence as there used to be. Although now there are two California serogroup viruses circulating in Finland, INKV is still more abundant.

California serogroup virus infections are most often subclinical; however, we showed that especially INKV can cause severe disease in children. Like LACV infections in children, CNS disease was observed and required hospitalization. Yet the severe cases were rare. For the first time, we identified CHATV as a causative agent of summertime febrile disease in humans. In contrast to acute INKV infection, CHATV infection was observed in adults. Both INKV and CHATV caused fever together with sore throat, nausea and vomiting and neurological symptoms including disorientation, nuchal rigidity, headache and/or drowsiness. All these symptoms are similar to those described in other California serogroup virus infections.

The incidence of INKV infection in children requires further studies. In addition, more detailed information about the CHATV geographic distribution as well as the frequency of both viruses in mosquitoes would be valuable. These viruses circulate in the same areas and evidently cause disease in humans. However, no isolates have been recovered from infected humans. These human virus isolates could be useful in understanding why so few clinical human cases appear and, when they do, why they manifest as CNS disease.

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References

Bahig (BAH) strain. Eg B 90, 1970a, *The American Journal of Tropical Medicine and Hygiene,* 19, 6, Suppl:1147-8.

Juan Diaz (JD) strain. MARU 8563, 1970b, *The American Journal of Tropical Medicine and Hygiene,* 19, 6, Suppl:1149-50.

Lokern (LOK) strain. FMS 4332, 1970c, *The American Journal of Tropical Medicine and Hygiene,* 19, 6, Suppl:1111-2.

Tete (TETE) strain. AN 3518, 1970d, *The American Journal of Tropical Medicine and Hygiene,* 19, 6, Suppl:1159-60.

Trubanaman (TRU) strain. MRM 3630, 1970e, *The American Journal of Tropical Medicine and Hygiene,* 19, 6, Suppl:1101-2.

Amundson, T.E. & Yuill, T.M. 1981, Natural La Crosse virus infection in the red fox (Vulpes fulva), gray fox (urocyon cinereoargenteus), raccoon (Procyon lotor), and opossum (Didelphis virginiana), *The American Journal of Tropical Medicine and Hygiene,* 30, 3, 706-714.

Anderson, C.R., Aitken, T.H., Spence, L.P. & Downs, W.G. 1960, Kairi virus, a new virus from Trinidadian forest mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 9, 70-72.

Anderson, C.R., Spence, L., Downs, W.G. & Aitken, T.H. 1961, Oropouche virus: a new human disease agent from Trinidad, West Indies, *The American Journal of Tropical Medicine and Hygiene,* 10, 574-578.

Anderson, C.R., Spence, L.P., Downs, W.G. & Aitken, T.H. 1960, Manzanilla virus: a new virus isolated from the blood of a howler monkey in Trinidad, W.I, *The American Journal of Tropical Medicine and Hygiene,* 9, 78-80.

Anderson, J.F., Main, A.J., Armstrong, P.M., Andreadis, T.G. & Ferrandino, F.J. 2015, Arboviruses in North Dakota, 2003-2006, *The American Journal of Tropical Medicine and Hygiene,* 92, 2, 377-393.

Andreadis, T.G., Anderson, J.F., Armstrong, P.M. & Main, A.J. 2008, Isolations of Jamestown Canyon virus (Bunyaviridae: Orthobunyavirus) from field-collected mosquitoes (Diptera: Culicidae) in Connecticut, USA: a ten-year analysis, 1997-2006, *Vector borne and zoonotic diseases (Larchmont, N.Y.),* 8, 2, 175-188.

Andreadis, T.G., Armstrong, P.M., Anderson, J.F. & Main, A.J. 2014, Spatial-temporal analysis of Cache Valley virus (Bunyaviridae: Orthobunyavirus) infection in anopheline and culicine mosquitoes (Diptera: Culicidae) in the northeastern United States, 1997-2012, *Vector borne and zoonotic diseases (Larchmont, N.Y.),* 14, 10, 763-773.

Andrews, W.N., Rowley, W.A., Wong, Y.W., Dorsey, D.C. & Hausler, W.J.,Jr 1977, Isolation of trivittatus virus from larvae and adults reared from field-collected larvae of Aedes trivittatus (Diptera: Culicidae), *Journal of medical entomology,* 13, 6, 699-701.

Artsob, H. 1985, Arbovirus activity in Canada in 1984, *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne,* 133, 10, 1026.

Artsob, H., Spence, L., Caughey, W.C. & Wherrett, J.R. 1981, Aseptic meningitis in Ontario, *Canadian Medical Association journal,* 125, 9, 958, 962.

Bakonyi, T., Kolodziejek, J., Rudolf, I., Bercic, R., Nowotny, N. & Hubalek, Z. 2013, Partial genetic characterization of Sedlec virus (Orthobunyavirus, Bunyaviridae), *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* 19, 244-249.

Balducci, M., Verani, P., Lopes, M.C. & Gregorig, B. 1973, Isolation in italy of bahig and matruh viruses (tete group) from migratory birds, *Ann. Microbiol.,* 124b, 231-237.

Balfour, H.H.,Jr, Siem, R.A., Bauer, H. & Quie, P.G. 1973, California arbovirus (La Crosse) infection I. Clinical and laboratory findings in 66 children with meningoencephalitis, *Pediatrics,* 52, 5, 680-691.

Bardos, V. & Danielova, V. 1959, The Tahyna virus--a virus isolated from mosquitoes in Czechoslovakia, *Journal of hygiene, epidemiology, microbiology, and immunology,* 3, 264-276.

Bardos, V., Medek, M., Kania, V. & Hubalek, Z. 1975, Isolation of Tahyna virus from the blood of sick children, *Acta Virologica. English ed,* 19, 5, 447.

Bardos, V., Ryba, J. & Hubalek, Z. 1975, Isolation of Tahyna virus from field collected Culiseta annulata (Schrk.) larvae, *Acta Virologica. English ed,* 19, 5, 446.

Bastos Mde, S., Figueiredo, L.T., Naveca, F.G., Monte, R.L., Lessa, N., Pinto de Figueiredo, R.M., Gimaque, J.B., Pivoto Joao, G., Ramasawmy, R. & Mourao, M.P. 2012, Identification of Oropouche Orthobunyavirus in the cerebrospinal fluid of three patients in the Amazonas, Brazil, *The American Journal of Tropical Medicine and Hygiene,* 86, 4, 732-735.

Beaty, B.J., Casals, J., Brown, K.L., Gundersen, C.B., Nelson, D., McPherson, J.T. & Thompson, W.H. 1982, Indirect fluorescent-antibody technique for serological diagnosis of La Crosse (California) virus infections, *Journal of clinical microbiology,* 15, 3, 429-434.

Bellocq, C. & Kolakofsky, D. 1987, Translational requirement for La Crosse virus S-mRNA synthesis: a possible mechanism, *Journal of virology,* 61, 12, 3960-3967.

Bennett, R.S., Cress, C.M., Ward, J.M., Firestone, C.Y., Murphy, B.R. & Whitehead, S.S. 2008, La Crosse virus infectivity, pathogenesis, and immunogenicity in mice and monkeys, *Virology journal,* 5, 25-422X-5-25.

Bennett, R.S., Gresko, A.K., Murphy, B.R. & Whitehead, S.S. 2011a, Tahyna virus genetics, infectivity, and immunogenicity in mice and monkeys, *Virology journal,* 8, 135-422X-8-135.

Bennett, R.S., Nelson, J.T., Gresko, A.K., Murphy, B.R. & Whitehead, S.S. 2011b, The full genome sequence of three strains of Jamestown Canyon virus and their pathogenesis in mice or monkeys, *Virology journal,* 8, 136-422X-8-136.

Bernardes-Terzian, A.C., de-Moraes-Bronzoni, R.V., Drumond, B.P., Da Silva-Nunes, M., da-Silva, N.S., Urbano-Ferreira, M., Speranca, M.A. & Nogueira, M.L. 2009, Sporadic oropouche virus infection, acre, Brazil, *Emerging infectious diseases,* 15, 2, 348-350.

Bird, B.H., Ksiazek, T.G., Nichol, S.T. & Maclachlan, N.J. 2009, Rift Valley fever virus, *Journal of the American Veterinary Medical Association,* 234, 7, 883-893.

Blakqori, G., Delhaye, S., Habjan, M., Blair, C.D., Sanchez-Vargas, I., Olson, K.E., Attarzadeh-Yazdi, G., Fragkoudis, R., Kohl, A., Kalinke, U., Weiss, S., Michiels, T., Staeheli, P. & Weber, F. 2007, La Crosse bunyavirus nonstructural protein NSs serves to suppress the type I interferon system of mammalian hosts, *Journal of virology,* 81, 10, 4991-4999.

Blitvich, B.J., Saiyasombat, R., Dorman, K.S., Garcia-Rejon, J.E., Farfan-Ale, J.A. & Lorono-Pino, M.A. 2012a, Sequence and phylogenetic data indicate that an orthobunyavirus recently detected in the Yucatan Peninsula of Mexico is a novel reassortant of Potosi and Cache Valley viruses, *Archives of Virology,* 157, 6, 1199-1204.

Blitvich, B.J., Saiyasombat, R., Talavera-Aguilar, L.G., Garcia-Rejon, J.E., Farfan-Ale, J.A., Machain-Williams, C. & Lorono-Pino, M.A. 2012b, Orthobunyavirus antibodies in humans, Yucatan Peninsula, Mexico, *Emerging infectious diseases,* 18, 10, 1629-1632.

Blitvich, B.J., Saiyasombat, R., Travassos da Rosa, A., Tesh, R.B., Calisher, C.H., Garcia-Rejon, J.E., Farfan-Ale, J.A., Lorono, R.E., Bates, A. & Lorono-Pino, M.A. 2012c, Orthobunyaviruses, a common cause of infection of livestock in the Yucatan peninsula of Mexico, *The American Journal of Tropical Medicine and Hygiene,* 87, 6, 1132-1139.

Blitvich, B.J., Staley, M., Lorono-Pino, M.A., Garcia-Rejon, J.E., Farfan-Ale, J.A. & Dorman, K.S. 2012d, Identification of a novel subtype of South River virus (family Bunyaviridae), *Archives of Virology,* 157, 6, 1205-1209.

Bond, J.O., Hammon, W.M., Lewis, A.L., Sather, G.E. & Taylor, D.J. 1966, California group arboviruses in Florida and report of a new strain, Keystone virus, *Public health reports,* 81, 7, 607-613.

Boromisa, R.D. & Grayson, M.A. 1990, Incrimination of Aedes provocans as a vector of Jamestown Canyon virus in an enzootic focus of northeastern New York, *Journal of the American Mosquito Control Association,* 6, 3, 504-509.

Borucki, M.K., Kempf, B.J., Blitvich, B.J., Blair, C.D. & Beaty, B.J. 2002, La Crosse virus: replication in vertebrate and invertebrate hosts, *Microbes and infection / Institut Pasteur,* 4, 3, 341-350.

Bowen, M.D., Trappier, S.G., Sanchez, A.J., Meyer, R.F., Goldsmith, C.S., Zaki, S.R., Dunster, L.M., Peters, C.J., Ksiazek, T.G., Nichol, S.T. & RVF Task Force 2001, A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia, *Virology,* 291, 2, 185-190.

Briese, T., Bird, B., Kapoor, V., Nichol, S.T. & Lipkin, W.I. 2006, Batai and Ngari viruses: M segment reassortment and association with severe febrile disease outbreaks in East Africa, *Journal of virology,* 80, 11, 5627-5630.

Briese, T., Kapoor, V. & Lipkin, W.I. 2007, Natural M-segment reassortment in Potosi and Main Drain viruses: implications for the evolution of orthobunyaviruses, *Archives of Virology,* 152, 12, 2237-2247.

Briese, T., Rambaut, A. & Lipkin, W.I. 2004, Analysis of the medium (M) segment sequence of Guaroa virus and its comparison to other orthobunyaviruses, *The Journal of general virology,* 85, Pt 10, 3071-3077.

Brummer-Korvenkontio, M. 1973a, Arboviruses in Finland. V. Serological survey of antibodies against Inkoo virus (California group) in human, cow, reindeer, and wildlife sera, *The American Journal of Tropical Medicine and Hygiene,* 22, 5, 654-661.

Brummer-Korvenkontio, M. 1973b, Batai (Calovo) arbovirus neutralising antibodies in Finland, *Annales Medicinae Experimentalis et Biologiae Fenniae,* 51, 4, 158-161.

Brummer-Korvenkontio, M., Saikku, P., Korhonen, P., Ulmanen, I., Reunala, T. & Karvonen, J. 1973, Arboviruses in Finland. IV. Isolation and characterization of Inkoo virus, a Finnish representative of the California group, *The American Journal of Tropical Medicine and Hygiene,* 22, 3, 404-413.

Bryant, J.E., Crabtree, M.B., Nam, V.S., Yen, N.T., Duc, H.M. & Miller, B.R. 2005, Isolation of arboviruses from mosquitoes collected in northern Vietnam, *The American Journal of Tropical Medicine and Hygiene,* 73, 2, 470-473.

Buescher, E.L., Byrne, R.J., Clarke, G.C., Gould, D.J., Russell, P.K., Scheider, F.G. & Yuill, T.M. 1970, Cache Valley virus in the Del Mar Va Peninsula. I. Virologic and serologic evidence of infection, *The American Journal of Tropical Medicine and Hygiene,* 19, 3, 493-502.

Burgdorfer, W., Newhouse, V.F. & Thomas, L.A. 1961, Isolation of California encephalitis virus from the blood of a snowshoe hare (Lepus americanus) in western Montana, *American journal of hygiene,* 73, 344-349.

Butenko, A.M., Demikhov, V.G., Nedialkova, M.S. & Lavrova, N.A. 1995, Serodiagnosis and epidemiology of a California encephalitis group of infections in the Ryazan' region, *Voprosy virusologii,* 40, 1, 17-21.

Butenko, A.M., Vladimirtseva, E.A., Lvov, S.D., Calisher, C.H. & Karabatsos, N. 1991, California serogroup viruses from mosquitoes collected in the USSR, *The American Journal of Tropical Medicine and Hygiene,* 45, 3, 366-370.

Calisher, C.H. 1983, Taxonomy, classification, and geographic distribution of California serogroup bunyaviruses, *Progress in clinical and biological research,* 123, 1-16.

Calisher, C.H., Ahmann, S.J., Grimstad, P.R., Hamm, J.G. & Parsons, M.A. 1981, Distribution and prevalence of Mermet virus infections in the central United States, *The American Journal of Tropical Medicine and Hygiene,* 30, 2, 473-476.

Calisher, C.H. & Bailey, R.E. 1981, Serodiagnosis of La Crosse virus infections in humans, *Journal of clinical microbiology,* 13, 2, 344-350.

Calisher, C.H., Chappell, W.A., Maness, K.S., Lord, R.D. & Sudia, W.D. 1971, Isolations of Nepuyo virus strains from Honduras, 1967, *The American Journal of Tropical Medicine and Hygiene,* 20, 2, 331-337.

Calisher, C.H., Coimbra, T.L., Lopez Ode, S., Muth, D.J., Sacchetta Lde, A., Francy, D.B., Lazuick, J.S. & Cropp, C.B. 1983, Identification of new Guama and Group C serogroup bunyaviruses and an ungrouped virus from Southern Brazil, *The American Journal of Tropical Medicine and Hygiene,* 32, 2, 424-431.

Calisher, C.H., Francy, D.B., Smith, G.C., Muth, D.J., Lazuick, J.S., Karabatsos, N., Jakob, W.L. & McLean, R.G. 1986, Distribution of Bunyamwera serogroup viruses in North America, 1956-1984, *The American Journal of Tropical Medicine and Hygiene,* 35, 2, 429-443.

Calisher, C.H., Gutierrez, E., Francy, D.B., Alava, A., Muth, D.J. & Lazuick, J.S. 1983, Identification of hitherto unrecognized arboviruses from Ecuador: members of serogroups B, C, Bunyamwera, Patois, and Minatitlan, *The American Journal of Tropical Medicine and Hygiene,* 32, 4, 877-885.

Calisher, C.H., Kokernot, R.H., De Moore, J.F., Boyd, K.R., Hayes, J. & Chappell, W.A. 1969, Arbovirus studies in the Ohio-Mississippi Basin, 1964-1967. VI. Mermet: A Simbu-group arbovirus, *The American Journal of Tropical Medicine and Hygiene,* 18, 5, 779-788.

Calisher, C.H., Lazuick, J.S., Justines, G., Francy, D.B., Monath, T.P., Gutierrez, E., Sabattini, M.S., Bowen, G.S. & Jakob, W.L. 1981, Viruses isolated from Aedeomyia squamipennis mosquitoes collected in Panama, Ecuador, and Argentina: establishment of the Gamboa serogroup, *The American Journal of Tropical Medicine and Hygiene,* 30, 1, 219-223.

Calisher, C.H., Lazuick, J.S., Muth, D.J., de Souza Lopes, O., Crane, G.T., Elbel, R.E. & Shope, R.E. 1980, Antigenic relationships among Tacaiuma complex viruses of the Anopheles A serogroup (Bunyaviridae), *Bulletin of the Pan American Health Organization,* 14, 4, 386-391.

Calisher, C.H., Lazuick, J.S., Wolff, K.L. & Muth, D.J. 1984, Antigenic relationships among Turlock serogroup Bunyaviruses as determined by neutralization tests, *Acta virologica,* 28, 2, 148-151.

Calisher, C.H., McLean, R.G., Zeller, H.G., Francy, D.B., Karabatsos, N. & Bowen, R.A. 1990, Isolation of Tete serogroup bunyaviruses from Ceratopogonidae collected in Colorado, *The American Journal of Tropical Medicine and Hygiene,* 43, 3, 314-318.

Calisher, C.H., Monath, T.P., Sabattini, M.S., Mitchell, C.J., Lazuick, J.S., Tesh, R.B. & Cropp, C.B. 1987, A newly recognized vesiculovirus, Calchaqui virus, and subtypes of Melao and Maguari viruses from Argentina, with serologic evidence for infections of humans and horses, *The American Journal of Tropical Medicine and Hygiene,* 36, 1, 114-119.

Calisher, C.H., Oro, J.G., Lord, R.D., Sabattini, M.S. & Karabatsos, N. 1988, Kairi virus identified from a febrile horse in Argentina, *The American Journal of Tropical Medicine and Hygiene,* 39, 5, 519-521.

Calisher, C.H., Pretzman, C.I., Muth, D.J., Parsons, M.A. & Peterson, E.D. 1986, Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies, *Journal of clinical microbiology,* 23, 4, 667-671.

Calisher, C.H., Sabattini, M.S., Monath, T.P. & Wolff, K.L. 1988, Cross-neutralization tests among Cache Valley virus isolates revealing the existence of multiple subtypes, *The American Journal of Tropical Medicine and Hygiene,* 39, 2, 202-205.

Calisher, C.H., Sasso, D.R., Maness, K.S., Gheorghiu, V.N. & Shope, R.E. 1973, Relationships of anopheles A group arboviruses, *Proceedings of the Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.),* 143, 2, 465-468.

Campbell, G.L., Hardy, J.L., Eldridge, B.F. & Reeves, W.C. 1991, Isolation of Northway serotype and other Bunyamwera serogroup bunyaviruses from California and Oregon mosquitoes, 1969-1985, *The American Journal of Tropical Medicine and Hygiene,* 44, 6, 581-588.

Campbell, G.L., Mataczynski, J.D., Reisdorf, E.S., Powell, J.W., Martin, D.A., Lambert, A.J., Haupt, T.E., Davis, J.P. & Lanciotti, R.S. 2006, Second human case of Cache Valley virus disease, *Emerging infectious diseases,* 12, 5, 854-856.

Campbell, W.P. & Huang, C. 1999, Sequence comparisons of medium RNA segment among 15 California serogroup viruses, *Virus research,* 61, 2, 137-144. doi: S0168170299000337 [pii].

Carey, D.E., Reuben, R., George, S., Shope, R.E. & Myers, R.M. 1971, Kammavanpettai, Kannamangalam, Sembalam and Thimiri viruses: four unrelated new agents isolated from birds in India, *The Indian journal of medical research,* 59, 11, 1708-1711.

Carvalho, V.L., Nunes, M.R., da Silva, E.V., Vieira, C.M., Gomes, M., Casseb, S.M., Rodrigues, S.G., Nunes-Neto, J.P., Quaresma, J.A. & Vasconcelos, P.F. 2009, Genetic characterization of orthobunyavirus Melao, strains BE AR633512 and BE AR8033, and experimental infection in golden hamsters (Mesocricetus auratus), *The Journal of general virology,* 90, Pt 1, 223-233.

Causey, O.R., Causey, C.E., Maroja, O.M. & Macedo, D.G. 1961, The isolation of arthropod-borne viruses, including members of two hitherto undescribed serological groups, in the Amazon region of Brazil, *The American Journal of Tropical Medicine and Hygiene,* 10, 227-249.

Causey, O.R., Kemp, G.E., Causey, C.E. & Lee, V.H. 1972, Isolations of Simbu-group viruses in Ibadan, Nigeria 1964-69, including the new types Sango, Shamonda, Sabo and Shuni, *Annals of Tropical Medicine and Parasitology,* 66, 3, 357-362.

Centers for Disease Control and Prevention (CDC) 2011, Human Jamestown canyon virus infection - Montana, 2009, *MMWR.Morbidity and mortality weekly report,* 60, 20, 652-655.

Chamberlain, R.W., Sudia, W.D. & Coleman, P.H. 1969, Isolations of an arbovirus of the Bunyamwera group (Tensaw virus) from mosquitoes in the Southeastern United States, 1960-1963, *The American Journal of Tropical Medicine and Hygiene,* 18, 1, 92-97.

Chappell, W.A., Halonen, P.E., Toole, R.F., Calisher, C.H. & Chester, L. 1969, Preparation of La Crosse virus hemagglutinating antigen in BHK-21 suspension cell cultures, *Applied Microbiology,* 18, 3, 433-437.

Chernesky, M.A. 1968, Transmission of California encephalitis virus by mosquitoes, *Canadian journal of microbiology,* 14, 1, 19-23.

Chevalier, V., Pepin, M., Plee, L. & Lancelot, R. 2010, Rift Valley fever--a threat for Europe?, *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin,* 15, 10, 19506.

Chowdhary, R., Street, C., Travassos da Rosa, A., Nunes, M.R., Tee, K.K., Hutchison, S.K., Vasconcelos, P.F., Tesh, R.B., Lipkin, W.I. & Briese, T. 2012, Genetic characterization of the Wyeomyia group of orthobunyaviruses and their phylogenetic relationships, *The Journal of general virology,* 93, Pt 5, 1023-1034.

Chung, S.I., Livingston, C.W.,Jr, Edwards, J.F., Crandell, R.W., Shope, R.E., Shelton, M.J. & Collisson, E.W. 1990, Evidence that Cache Valley virus induces congenital malformations in sheep, *Veterinary microbiology,* 21, 4, 297-307.

Clarke, D.H. & Casals, J. 1958, Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses, *The American Journal of Tropical Medicine and Hygiene,* 7, 5, 561-573.

Coffey, L.L., Page, B.L., Greninger, A.L., Herring, B.L., Russell, R.C., Doggett, S.L., Haniotis, J., Wang, C., Deng, X. & Delwart, E.L. 2014, Enhanced arbovirus surveillance with deep sequencing: Identification of novel rhabdoviruses and bunyaviruses in Australian mosquitoes, *Virology,* 448, 146-158.

Coleman, P.H. 1969, Tensaw virus, a new member of the Bunyamwera arbovirus group from the Southern United States, *The American Journal of Tropical Medicine and Hygiene,* 18, 1, 81-91.

Coleman, P.H., Ryder, S. & Work, T.H. 1969, Mahogany hammock virus, a new Guama group arbovirus from the Florida everglades, *American Journal of Epidemiology,* 89, 2, 217-221.

Cybinski, D.H. 1984, Douglas and Tinaroo viruses: two Simbu group arboviruses infecting Culicoides brevitarsis and livestock in Australia, *Australian Journal of Biological Sciences,* 37, 3, 91-97.

De Souza Lopes, O., DE Abreu Sacchetta, L., Fonseca, I.E. & Lacerda, J.P. 1975, Bertioga (Guama group) and Anhembi (Bunyamwera group), two new arboviruses isolated in

Sao Paulo, Brazil, *The American Journal of Tropical Medicine and Hygiene,* 24, 1, 131-134.

DeFoliart, G.R., Anslow, R.O., Hanson, R.P., Morris, C.D., Papadopoulos, O. & Sather, G.E. 1969, Isolation of Jamestown Canyon serotype of California encephalitis virus from naturally infected Aedes mosquitoes and tabanids, *The American Journal of Tropical Medicine and Hygiene,* 18, 3, 440-447.

Demikhov, V.G. 1995, Outcomes and prognosis of diseases caused by Inkoo and Tahyna viruses, *Voprosy virusologii,* 40, 2, 72-74.

Demikhov, V.G. & Chaitsev, V.G. 1995, Neurologic characteristics of diseases caused by Inkoo and Tahyna viruses, *Voprosy virusologii,* 40, 1, 21-25.

Derodaniche, E., Paesdeandrade, A. & Galindo, P. 1964, Isolation of Two Antigenically Distinct Arthropod-Borne Viruses of Group C in Panama, *The American Journal of Tropical Medicine and Hygiene,* 13, 839-843.

Doherty, R.L., Carley, J.G., Filippich, C., Kay, B.H., Gorman, B.M. & Rajapaksa, N. 1977, Isolation of Sindbis (alphavirus) and Leanyer viruses from mosquitoes collected in the Northern Territory of Australia, 1974, *The Australian Journal of Experimental Biology and Medical Science,* 55, 4, 485-489.

Doherty, R.L., Carley, J.G., Kay, B.H., Filippich, C., Marks, E.N. & Frazier, C.L. 1979, Isolation of virus strains from mosquitoes collected in Queensland, 1972-1976, *The Australian Journal of Experimental Biology and Medical Science,* 57, 5, 509-520.

Doherty, R.L., Carley, J.G., Mackerras, M.J. & Marks, E.N. 1963, Studies of arthropod-borne virus infections in Queensland. III. Isolation and characterization of virus strains from wild-caught mosquitoes in North Queensland, *The Australian Journal of Experimental Biology and Medical Science,* 41, 17-39.

Doherty, R.L., Carley, J.G., Standfast, H.A., Dyce, A.L. & Snowdon, W.A. 1972, Virus strains isolated from arthropods during an epizootic of bovine ephemeral fever in Queensland, *Australian Veterinary Journal,* 48, 3, 81-86.

Doherty, R.L., Whitehead, R.H., Wetters, E.J., Gorman, B.M. & Carley, J.G. 1970, A survey of antibody to 10 arboviruses (Koongol group, Mapputta group and ungrouped) isolated in Queensland, *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 64, 5, 748-753.

Dunn, E.F., Pritlove, D.C. & Elliott, R.M. 1994, The S RNA genome segments of Batai, Cache Valley, Guaroa, Kairi, Lumbo, Main Drain and Northway bunyaviruses: sequence determination and analysis, *The Journal of general virology,* 75 (Pt 3), Pt 3, 597-608.

Ehrnst, A., Peters, C.J., Niklasson, B., Svedmyr, A. & Holmgren, B. 1985, Neurovirulent Toscana virus (a sandfly fever virus) in Swedish man after visit to Portugal, *Lancet,* 1, 8439, 1212-1213.

Eldridge, B.F., Glaser, C., Pedrin, R.E. & Chiles, R.E. 2001, The first reported case of California encephalitis in more than 50 years, *Emerging infectious diseases,* 7, 3, 451-452.

Elliott, R.M. 2014, Orthobunyaviruses: recent genetic and structural insights, *Nature reviews.Microbiology,* 12, 10, 673-685.

Elliott, R.M. & Blakqori, G. 2011, *Molecular Biology of Orthobunyaviruses in book Bunyaviridae: Molecular and Cellular Biology,* Caister Academic Press.

Embil, J.A., Camfield, P.R. & Artsob, H. 1985, California encephalitis in New Brunswick, *Canadian Medical Association journal,* 132, 10, 1166.

Embil, J.A., Camfield, P.R., Artsob, H. & Rozee, K.R. 1982, California encephalitis in Nova Scotia, *Canadian Medical Association journal,* 127, 10, 957, 959.

Emmons, R.W., Woodie, J.D., Laub, R.L. & Oshiro, L.S. 1983, Main Drain virus as a cause of equine encephalomyelitis, *Journal of the American Veterinary Medical Association,* 183, 5, 555-558.

Eriksson, M., Holmgren, L., Janlert, U., Jansson, J.H., Lundblad, D., Stegmayr, B., Soderberg, S. & Eliasson, M. 2011, Large improvements in major cardiovascular risk factors in the population of northern Sweden: the MONICA study 1986-2009, *Journal of internal medicine,* 269, 2, 219-231.

Fauvel, M., Artsob, H., Calisher, C.H., Davignon, L., Chagnon, A., Skvorc-Ranko, R. & Belloncik, S. 1980, California group virus encephalitis in three children from Quebec: clinical and serologic findings, *Canadian Medical Association journal,* 122, 1, 60-2, 64.

Fazakerley, J.K., Gonzalez-Scarano, F., Strickler, J., Dietzschold, B., Karush, F. & Nathanson, N. 1988, Organization of the middle RNA segment of snowshoe hare Bunyavirus, *Virology,* 167, 2, 422-432.

Feng, Y., Fu, S.H., Yang, W.H., Zhang, Y.Z., He, B., Tu, C.C., Liang, G.D. & Zhang, H.L. 2015, Isolation and full-length genome analysis of mosquito-borne Manzanilla virus from Yunnan Province, China, *BMC research notes,* 8, 255-015-1198-5.

Fields, B.N., Henderson, B.E., Coleman, P.H. & Work, T.H. 1969, Pahayokee and Shark River, two new arboviruses related to Patois and Zegla from the Florida everglades, *American Journal of Epidemiology,* 89, 2, 222-226.

Fischer, M., Schirrmeier, H., Wernike, K., Wegelt, A., Beer, M. & Hoffmann, B. 2013, Development of a pan-Simbu real-time reverse transcriptase PCR for the detection of Simbu serogroup viruses and comparison with SBV diagnostic PCR systems, *Virology journal,* 10, 327-422X-10-327.

Francy, D.B., Jaenson, T.G., Lundstrom, J.O., Schildt, E.B., Espmark, A., Henriksson, B. & Niklasson, B. 1989, Ecologic studies of mosquitoes and birds as hosts of Ockelbo virus in Sweden and isolation of Inkoo and Batai viruses from mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 41, 3, 355-363.

Francy, D.B., Karabatsos, N., Wesson, D.M., Moore, C.G.,Jr, Lazuick, J.S., Niebylski, M.L., Tsai, T.F. & Craig, G.B.,Jr 1990, A new arbovirus from Aedes albopictus, an Asian mosquito established in the United States, *Science (New York, N.Y.),* 250, 4988, 1738-1740.

Fulhorst, C.F., Hardy, J.L., Eldridge, B.F., Chiles, R.E. & Reeves, W.C. 1996, Ecology of Jamestown Canyon virus (Bunyaviridae: California serogroup) in coastal California, *The American Journal of Tropical Medicine and Hygiene,* 55, 2, 185-189.

Gard, G., Marshall, I.D. & Woodroofe, G.M. 1973, Annually recurrent epidemic polyarthritis and Ross River virus activity in a coastal area of New South Wales. II. Mosquitoes, viruses, and wildlife, *The American Journal of Tropical Medicine and Hygiene,* 22, 4, 551-560.

Garry, C.E. & Garry, R.F. 2004, Proteomics computational analyses suggest that the carboxyl terminal glycoproteins of Bunyaviruses are class II viral fusion protein (beta-penetrenes), *Theoretical biology & medical modelling,* 1, 10.

Gauci, P.J., McAllister, J., Mitchell, I.R., Boyle, D.B., Bulach, D.M., Weir, R.P., Melville, L.F. & Gubala, A.J. 2015, Genomic characterisation of three Mapputta group viruses, a serogroup of Australian and Papua New Guinean bunyaviruses associated with human disease, *PloS one,* 10, 1, e0116561.

Gauld, L.W., Yuill, T.M., Hanson, R.P. & Sinha, S.K. 1975, Isolation of La Crosse virus (California encephalitis group) from the chipmunk (Tamias striatus), an amplifier host, *The American Journal of Tropical Medicine and Hygiene,* 24, 6 Pt 1, 999-1005.

Gerhardt, R.R., Gottfried, K.L., Apperson, C.S., Davis, B.S., Erwin, P.C., Smith, A.B., Panella, N.A., Powell, E.E. & Nasci, R.S. 2001, First isolation of La Crosse virus from naturally infected Aedes albopictus, *Emerging infectious diseases,* 7, 5, 807-811.

Gerrard, S.R., Li, L., Barrett, A.D. & Nichol, S.T. 2004, Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa, *Journal of virology,* 78, 16, 8922-8926.

Gibbs, C.J.,Jr, Bruckner, E.A. & Schenker, S. 1964, A Case of Apeu Virus Infection, *The American Journal of Tropical Medicine and Hygiene,* 13, 108-113.

Goff, G., Whitney, H. & Drebot, M.A. 2012, Roles of host species, geographic separation, and isolation in the seroprevalence of Jamestown Canyon and snowshoe hare viruses in Newfoundland, *Applied and Environmental Microbiology,* 78, 18, 6734-6740.

Goller, K.V., Hoper, D., Schirrmeier, H., Mettenleiter, T.C. & Beer, M. 2012, Schmallenberg virus as possible ancestor of Shamonda virus, *Emerging infectious diseases,* 18, 10, 1644-1646. doi: 10.3201/eid1810.120835 [doi].

Gonzalez-Scarano, F., Pobjecky, N. & Nathanson, N. 1984, La Crosse bunyavirus can mediate pH-dependent fusion from without, *Virology,* 132, 1, 222-225.

Gonzalez-Scarano, F., Shope, R.E., Calisher, C.E. & Nathanson, N. 1982, Characterization of monoclonal antibodies against the G1 and N proteins of LaCrosse and Tahyna, two California serogroup bunyaviruses, *Virology,* 120, 1, 42-53.

Grimstad, P.R., Calisher, C.H., Harroff, R.N. & Wentworth, B.B. 1986, Jamestown Canyon virus (California serogroup) is the etiologic agent of widespread infection in Michigan humans, *The American Journal of Tropical Medicine and Hygiene,* 35, 2, 376-386.

Grimstad, P.R., Shabino, C.L., Calisher, C.H. & Waldman, R.J. 1982, A case of encephalitis in a human associated with a serologic rise to Jamestown Canyon virus, *The American Journal of Tropical Medicine and Hygiene,* 31, 6, 1238-1244.

Groot, H., Oya, A., Bernal, C. & Barreto-Reyes, P. 1959, Guaroa virus, a new agent isolated in Colombia, South America, *The American Journal of Tropical Medicine and Hygiene,* 8, 604-609.

Groseth, A., Mampilli, V., Weisend, C., Dahlstrom, E., Porcella, S.F., Russell, B.J., Tesh, R.B. & Ebihara, H. 2014, Molecular Characterization of Human Pathogenic Bunyaviruses of the Nyando and Bwamba/Pongola Virus Groups Leads to the Genetic Identification of Mojui dos Campos and Kaeng Khoi Virus, *PLoS neglected tropical diseases,* 8, 9, 3147.

Groseth, A., Vine, V., Weisend, C. & Ebihara, H. 2015, Complete genome sequence of trivittatus virus, *Archives of Virology,* 160, 2637-9.

Hacker, D., Raju, R. & Kolakofsky, D. 1989, La Crosse virus nucleocapsid protein controls its own synthesis in mosquito cells by encapsidating its mRNA, *Journal of virology,* 63, 12, 5166-5174.

Hacker, J.K. & Hardy, J.L. 1997, Adsorptive endocytosis of California encephalitis virus into mosquito and mammalian cells: a role for G1, *Virology,* 235, 1, 40-47.

Haddow, A.D. & Odoi, A. 2009, The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007, *PloS one,* 4, 7, e6145.

Hammon, W.M. & Reeves, W.C. 1952, California encephalitis virus, a newly described agent. Part l.Evidence of Natural Infection in Man and Other Animals, *California medicine,* 77, 5, 303-309.

Hammon, W.M., Reeves, W.C. & Sather, G. 1952, California encephalitis virus, a newly described agent. II. Isolations and attempts to identify and characterize the agent, *Journal of immunology (Baltimore, Md.: 1950),* 69, 5, 493-510.

Hammon, W.M. & Sather, G. 1966, History and recent reappearance of viruses in the California encephalitis group, *The American Journal of Tropical Medicine and Hygiene,* 15, 2, 199-204.

Hang, J., Forshey, B.M., Yang, Y., Solorzano, V.F., Kuschner, R.A., Halsey, E.S., Jarman, R.G. & Kochel, T.J. 2014, Genomic characterization of group C Orthobunyavirus reference strains and recent South American clinical isolates, *PloS one,* 9, 3, e92114.

Hayes, R.O., LaMotte, L.C. & Holden, P. 1967, Ecology of arboviruses in Hale County, Texas, during 1965, *The American Journal of Tropical Medicine and Hygiene,* 16, 5, 675-687.

Heath, S.E., Artsob, H., Bell, R.J. & Harland, R.J. 1989, Equine encephalitis caused by snowshoe hare (California serogroup) virus, *The Canadian veterinary journal.La revue veterinaire canadienne,* 30, 8, 669-671.

Henderson, B.E., Calisher, C.H., Coleman, P.H., Fields, B.N. & Work, T.H. 1969, Gumbo Limbo, a new group C arbovirus from the Florida everglades, *American Journal of Epidemiology,* 89, 2, 227-231.

Henderson, B.E., McCrae, A.W., Kirya, B.G., Ssenkubuge, Y. & Sempala, S.D. 1972, Arbovirus epizootics involving man, mosquitoes and vertebrates at Lunyo, Uganda 1968, *Annals of Tropical Medicine and Parasitology,* 66, 3, 343-355.

Hoffmann, B., Scheuch, M., Hoper, D., Jungblut, R., Holsteg, M., Schirrmeier, H., Eschbaumer, M., Goller, K.V., Wernike, K., Fischer, M., Breithaupt, A., Mettenleiter, T.C. & Beer, M. 2012, Novel orthobunyavirus in Cattle, Europe, 2011, *Emerging infectious diseases,* 18, 3, 469-472.

Holden, P. & Hess, A.D. 1959, Cache Valley virus, a previously undescribed mosquito-borne agent, *Science (New York, N.Y.),* 130, 3383, 1187-1188.

Hollidge, B.S., Nedelsky, N.B., Salzano, M.V., Fraser, J.W., Gonzalez-Scarano, F. & Soldan, S.S. 2012, Orthobunyavirus entry into neurons and other mammalian cells occurs via clathrin-mediated endocytosis and requires trafficking into early endosomes, *Journal of virology,* 86, 15, 7988-8001.

Hontz, R.D., Guevara, C., Halsey, E.S., Silvas, J., Santiago, F.W., Widen, S.G., Wood, T.G., Casanova, W., Vasilakis, N., Watts, D.M., Kochel, T.J., Ebihara, H. & Aguilar, P.V. 2015, Itaya virus, a Novel Orthobunyavirus Associated with Human Febrile Illness, Peru, *Emerging infectious diseases,* 21, 5, 781-788.

Huang, C., Shope, R.E., Spargo, B. & Campbell, W.P. 1996, The S RNA genomic sequences of Inkoo, San Angelo, Serra do Navio, South River and Tahyna bunyaviruses, *The Journal of general virology,* 77, Pt 8, 1761-1768.

Huang, C., Thompson, W.H., Karabatsos, N., Grady, L. & Campbell, W.P. 1997, Evidence that fatal human infections with La Crosse virus may be associated with a narrow range of genotypes, *Virus research,* 48, 2, 143-148.

Hubalek, Z. 2008, Mosquito-borne viruses in Europe, *Parasitology research,* 103 Suppl 1, S29-43.

Hubalek, Z., Juricova, Z., Halouzka, J., Butenko, A.M., Kondrasina, N.G., Guscina, E.A. & Morozova, T.N. 1990, Isolation and characterization of Sedlec virus, a new bunyavirus from birds, *Acta virologica,* 34, 4, 339-345.

Hubalek, Z. & Rudolf, I. 2012, Tick-borne viruses in Europe, *Parasitology research,* 111, 1, 9-36.

Hunt, A.R. & Calisher, C.H. 1979, Relationships of bunyamwera group viruses by neutralization, *The American Journal of Tropical Medicine and Hygiene,* 28, 4, 740-749.

Issel, C.J., Hoff, G.L. & Trainer, D.O. 1973, SEROLOGIC EVIDENCE OF INFECTION OF WHITE-TAILED DEER IN TEXAS WITH THREE CALIFORNIA GROUP ARBOVIRUSES, (JAMESTOWN CANYON, SAN ANGELO, AND KEYSTONE), *Journal of wildlife diseases,* 9, 3, 245-248.

Iversson, L.B., Travassos da Rosa, A.P., Coimbra, T.L., Ferreira, I.B. & Nassar Eda, S. 1987, Human disease in Ribeira Valley, Brazil caused by Caraparu, a group C arbovirus--report of a case, *Revista do Instituto de Medicina Tropical de Sao Paulo,* 29, 2, 112-117.

Jackson, B.T., Brewster, C.C. & Paulson, S.L. 2012, La Crosse virus infection alters blood feeding behavior in Aedes triseriatus and Aedes albopictus (Diptera: Culicidae), *Journal of medical entomology,* 49, 6, 1424-1429.

Johnson, R.T. 1983, Pathogenesis of La Crosse virus in mice, *Progress in clinical and biological research,* 123, 139-144.

Jonkers, A.H., Metselaar, D., de Andrade, A.H. & Tikasingh, E.S. 1967, Restan virus, a new group C arbovirus from Trinidad and Surinam, *The American Journal of Tropical Medicine and Hygiene,* 16, 1, 74-78.

Jonkers, A.H., Spence, L., Downs, W.G., Aitken, T.H. & Tikasingh, E.S. 1968a, Arbovirus studies in Bush Bush forest, Trinidad, W. I., September 1959-December 1964. V. Virus isolations, *The American Journal of Tropical Medicine and Hygiene,* 17, 2, 276-284.

Jonkers, A.H., Spence, L., Downs, W.G., Aitken, T.H. & Worth, C.B. 1968b, Arobovirus studies in Bush Bush Forest, Trinidad, W. I., September 1959-December 1964. VI. Rodent-associated viruses (VEE and agents of groups C and Guama): isolations and further studies, *The American Journal of Tropical Medicine and Hygiene,* 17, 2, 285-298.

Jöst, H., Bialonski, A., Schmetz, C., Günther, S., Becker, N. & Schmidt-Chanasit, J. 2011, Isolation and Phylogenetic Analysis of Batai Virus, Germany, *The American Journal of Tropical Medicine and Hygiene,* 84, 2, 241-243.

Kalunda, M., Lwanga-Ssozi, C., Lule, M. & Mukuye, A. 1985, Isolation of Chikungunya and Pongola viruses from patients in Uganda, *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 79, 4, 567.

Kappus, K.D., Monath, T.P., Kaminski, R.M. & Calisher, C.H. 1983, Reported encephalitis associated with California serogroup virus infections in the United States, 1963-1981, *Progress in clinical and biological research,* 123, 31-41.

Karabatsos, N. 1985, International catalogue of arboviruses, including certain other viruses of vertebrates, 3, 1-1147.

Kettyls, G.D., Verrall, V.M., Wilton, L.D., Clapp, J.B., Clarke, D.A. & Rublee, J.D. 1972, Arbovirus infections in man in British Columbia, *Canadian Medical Association journal,* 106, 11, 1175-1179.

King, A.M.Q., Lefkowitz, E., Adams, M.J. & Carstens, E.B. 2012, Virus taxonomy: classification and nomenclature of viruses: Ninth Report of the International Committee on Taxonomy of Viruses: Family - Bunyaviridae i *Virus Taxonomy*, red. Andrew M.Q. King, Elliot Lefkowitz, Michael J.Adams & Eric B. Carstens, Elsevier, San Diego, 725-741.

Kinney, R.M. & Calisher, C.H. 1981, Antigenic relationships among Simbu serogroup (Bunyaviridae) viruses, *The American Journal of Tropical Medicine and Hygiene,* 30, 6, 1307-1318.

Klimas, R.A., Ushijima, H., Clerx-Van Haaster, C.M. & Bishop, D.H. 1981, Radioimmune assays and molecular studies that place Anopheles B and Turlock serogroup viruses in the Bunyavirus genus (Bunyaviridae), *The American Journal of Tropical Medicine and Hygiene,* 30, 4, 876-887.

Kokernot, R.H., McIntosh, B.M., Worth, C.B., De, M.O.R.A.I.S.T. & Weinbren, M.P. 1962, Isolation of viruses from mosquitoes collected at Lumbo, Mozambique. I. Lumbo virus, a new virus isolated from Aedes (Skusea) pembaensis Theobald, *The American Journal of Tropical Medicine and Hygiene,* 11, 678-682.

Kokernot, R.H., Smithburn, K.C., De Meillon, B. & Paterson, H.E. 1958, Isolation of Bunyamwera virus from a naturally infected human being and further isolations from Aedes (Banksinella) circumluteolus theo, *The American Journal of Tropical Medicine and Hygiene,* 7, 6, 579-584.

Kokernot, R.H., Smithburn, K.C., Paterson, H.E. & McIntosh, B.M. 1960, Isolation of Germiston virus, a hitherto unknown agent, from culicine mosquitoes, and a report of infection in two laboratory workers, *The American Journal of Tropical Medicine and Hygiene,* 9, 62-69.

Kokernot, R.H., Smithburn, K.C., Weinbren, M.P. & De Meillon, B. 1957, Studies on arthropod-borne viruses of Tongaland. VI. Isolation of Pongola virus from Aedes (Banksinella) circumluteolus Theo, *The South African journal of medical sciences,* 22, 2-3, 81-92.

Kokernot, R.H., Szlamp, E.L., Levitt, J. & McIntosh, B.M. 1965, Survey for antibodies against arthropod-borne viruses in the sera of indigenous residents of the Caprivi Strip and Bechuanaland Protectorate, *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 59, 5, 553-562.

Kono, Y., Yusnita, Y., Mohd Ali, A.R., Maizan, M., Sharifah, S.H., Fauzia, O., Kubo, M. & Aziz, A.J. 2002, Characterization and identification of Oya virus, a Simbu serogroup virus of the genus Bunyavirus, isolated from a pig suspected of Nipah virus infection, *Archives of Virology,* 147, 8, 1623-1630.

Ksiazek, T.G. & Yuill, T.M. 1977, Viremia and antibody response to La Crosse virus in sentinel gray squirrels (Sciuris carolinensis) and chipmunks Tamias striatus), *The American Journal of Tropical Medicine and Hygiene,* 26, 4, 815-821.

Kuno, G., Mitchell, C.J., Chang, G.J. & Smith, G.C. 1996, Detecting bunyaviruses of the Bunyamwera and California serogroups by a PCR technique, *Journal of clinical microbiology,* 34, 5, 1184-1188.

Laloy, E., Breard, E., Sailleau, C., Viarouge, C., Desprat, A., Zientara, S., Klein, F., Hars, J. & Rossi, S. 2014, Schmallenberg virus infection among red deer, France, 2010-2012, *Emerging infectious diseases,* 20, 1, 131-134.

Lambert, A.J., Fryxell, R.T., Freyman, K., Ulloa, A., Velez, J.O., Paulsen, D., Lanciotti, R.S. & Moncayo, A. 2015, Comparative sequence analyses of La Crosse virus strain isolated from patient with fatal encephalitis, Tennessee, USA, *Emerging infectious diseases,* 21, 5, 833-836.

Lambert, A.J., Nasci, R.S., Cropp, B.C., Martin, D.A., Rose, B.C., Russell, B.J. & Lanciotti, R.S. 2005, Nucleic acid amplification assays for detection of La Crosse virus RNA, *Journal of clinical microbiology,* 43, 4, 1885-1889.

Lavrent'ev, M.V., Prilipov, A.G., L'vov, S.D. & L'vov, D.K. 2008, Phylogenetic analysis of the nucleotide sequences of Chatanga virus strains, the new representative of California encephalitis serocomplex, isolated in different regions of the Russian Federation, *Voprosy virusologii,* 53, 6, 25-29.

LeDuc, J.W. 1987, Epidemiology and ecology of the California serogroup viruses, *The American Journal of Tropical Medicine and Hygiene,* 37, 3 Suppl, 60S-68S.

LeDuc, J.W., Burger, J.F., Eldridge, B.F. & Russell, P.K. 1975, Ecology of Keystone virus, a transovarially maintained arbovirus, *Annals of the New York Academy of Sciences,* 266, 144-151.

Lennette, E.H., Ota, M.I., Fujimoto, F.Y., Wiener, A. & Loomis, E.C. 1957, Turlock virus: a presumably new arthropod-borne virus; isolation and identification, *The American Journal of Tropical Medicine and Hygiene,* 6, 6, 1024-1035.

Lewis, A.L., Hammon, W.M., Sather, G.E., Taylor, D.J. & Bond, J.O. 1965, Isolation of a California Group Arbovirus from Florida Mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 14, 451-455.

Linden, A., Desmecht, D., Volpe, R., Wirtgen, M., Gregoire, F., Pirson, J., Paternostre, J., Kleijnen, D., Schirrmeier, H., Beer, M. & Garigliany, M.M. 2012, Epizootic spread of Schmallenberg virus among wild cervids, Belgium, Fall 2011, *Emerging infectious diseases,* 18, 12, 2006-2008.

Liu, H., Shao, X.Q., Hu, B., Zhao, J.J., Zhang, L., Zhang, H.L., Bai, X., Zhang, R.X., Niu, D.Y., Sun, Y.G. & Yan, X.J. 2014, Isolation and complete nucleotide sequence of a Batai virus strain in Inner Mongolia, China, *Virology journal,* 11, 138-422X-11-138.

Lopes Ode, S., Forattini, O.P., Fonseca, I.E., Lacerda, J.P., Sacchetta, L.A. & Rabello, E.X. 1966, Boraceia virus. A new virus related to anopheles B virus, *Proceedings of the*

Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.), 123, 2, 502-504.

Lopez-Montero, N. & Risco, C. 2011, Self-protection and survival of arbovirus-infected mosquito cells, *Cellular microbiology,* 13, 2, 300-315.

Lu, Z., Fu, S., Wang, F., Nasci, R.S., Tang, Q. & Liang, G. 2011, Circulation of Diverse Genotypes of Tahyna Virus in Xinjiang, People's Republic of China, *The American Journal of Tropical Medicine and Hygiene,* 85, 3, 442-445.

Lu, Z., Lu, X., Fu, S., Zhang, S., Li, Z., Yao, X., Feng, Y., Lambert, A.J., Ni, D., Wang, F., Tong, S., Nasci, R.S., Feng, Y., Dong, Q., Zhai, Y., Gao, X., Wang, H., Tang, Q. & Liang, G. 2009, Tahyna Virus and Human Infection, China, *Emerging Infectious Diseases,* 15, 2, 306-309.

Ludwig, G.V., Israel, B.A., Christensen, B.M., Yuill, T.M. & Schultz, K.T. 1991, Role of La Crosse virus glycoproteins in attachment of virus to host cells, *Virology,* 181, 2, 564-571.

Lutwama, J.J., Rwaguma, E.B., Nawanga, P.L. & Mukuye, A. 2002, Isolations of Bwamba virus from south central Uganda and north eastern Tanzania, *African health sciences,* 2, 1, 24-28.

Lynch, J.A., Binnington, B.D. & Artsob, H. 1985, California serogroup virus infection in a horse with encephalitis, *Journal of the American Veterinary Medical Association,* 186, 4, 389.

Madoff, D.H. & Lenard, J. 1982, A membrane glycoprotein that accumulates intracellularly: cellular processing of the large glycoprotein of LaCrosse virus, *Cell,* 28, 4, 821-829.

Mahy, B.W.J. 2001, *A Dictionary of Virology,* Elsevier

Malkova, D. 1972, Yaba 1 virus in Czechoslovakia. I. Some physical and chemical properties of yaba 1 (Lednice 110) virus, *Acta virologica,* 16, 3, 264-266.

Malkova, D., Holubova, J., Cerny, V., Daniel, M., Fernandez, A., de la Cruz, J., Herrera, M. & Calisher, C.H. 1985, Estero real virus: a new virus isolated from argasid ticks Ornithodoros tadaridae in Cuba, *Acta virologica,* 29, 3, 247-250.

Mangiafico, J.A., Sanchez, J.L., Figueiredo, L.T., LeDuc, J.W. & Peters, C.J. 1988, Isolation of a newly recognized Bunyamwera serogroup virus from a febrile human in Panama, *The American Journal of Tropical Medicine and Hygiene,* 39, 6, 593-596.

Marchette, N.J. 1994, *Handbook of Zoonoses, Second Edition: Viral Zoonoses. Arboviral zoonoses of Asia,* Second Edition uppl., CRC Press

Marczinke, B.I. & Nichol, S.T. 2002, Nairobi sheep disease virus, an important tick-borne pathogen of sheep and goats in Africa, is also present in Asia, *Virology,* 303, 1, 146-151.

Matsumori, Y., Inai, K., Yanase, T., Ohashi, S., Kato, T., Yoshida, K. & Tsuda, T. 2002, Serological and genetic characterization of newly isolated Peaton virus in Japan. Brief report, *Archives of Virology,* 147, 2, 401-410.

McFarlane, B.L., Embil, J.A., Artsob, H., Spence, L. & Rozee, K.R. 1981a, Antibodies to the California group of arboviruses in the moose (Alces alces americana Clinton) population of Nova Scotia, *Canadian journal of microbiology,* 27, 11, 1219-1223.

McFarlane, B.L., Embree, J.E., Embil, J.A., Artsob, H., Weste, J.B. & Rozee, K.R. 1981b, Antibodies to snowshoe hare virus of the California group in the snowshoe hare (Lepus americanus) and domestic animal populations of Prince Edward Island, *Canadian journal of microbiology,* 27, 11, 1224-1227.

McIntosh, B.M., McGillivray, G.M. & Dickinson, D.B. 1965, Ingwavuma virus: an arbovirus isolated in South Africa, *The South African journal of medical sciences,* 30, 3, 67-70.

McJunkin, J.E., de los Reyes, E.C., Irazuzta, J.E., Caceres, M.J., Khan, R.R., Minnich, L.L., Fu, K.D., Lovett, G.D., Tsai, T. & Thompson, A. 2001, La Crosse encephalitis in children, *The New England journal of medicine,* 344, 11, 801-807.

McLean, D.M. 1983, Yukon isolates of snowshoe hare virus, 1972-1982, *Progress in clinical and biological research,* 123, 247-256.

McLean, R.G., Calisher, C.H. & Parham, G.L. 1987, Isolation of Cache Valley virus and detection of antibody for selected arboviruses in Michigan horses in 1980, *American Journal of Veterinary Research,* 48, 7, 1039-1041.

McLean, R.G., Kirk, L.J., Shriner, R.B., Cook, P.D., Myers, E.E., Gill, J.S. & Campos, E.G. 1996, The role of deer as a possible reservoir host of potosi virus, a newly recognized arbovirus in the United States, *Journal of wildlife diseases,* 32, 3, 444-452.

Mclintock, J., Curry, P.S., Wagner, R.J., Leung, M.K. & Iversen, J.O. 1976, Isolation of snowshoe hare virus from aedes implicatus larvae in saskatchewan canada, *Mosquito News,* 36(3), 233-237.

Meier-Stephenson, V., Langley, J.M., Drebot, M. & Artsob, H. 2007, Encephalitis in the summer: a case of snowshoe hare (California serogroup) virus infection in Nova Scotia, *Canada communicable disease report = Releve des maladies transmissibles au Canada,* 33, 11, 23-26.

Metselaar, D. 1966, Isolation of arboviruses of group A and group C in Surinam, *Tropical and geographical medicine,* 18, 2, 137-142.

Miller, B.R., DeFoliart, G.R. & Yuill, T.M. 1979, Aedes triseriatus and La Crosse virus: lack of infection in eggs of the first ovarian cycle following oral infection of females, *The American Journal of Tropical Medicine and Hygiene,* 28, 5, 897-901.

Mitchell, C.J., Lvov, S.D., Savage, H.M., Calisher, C.H., Smith, G.C., Lvov, D.K. & Gubler, D.J. 1993, Vector and host relationships of California serogroup viruses in western Siberia, *The American Journal of Tropical Medicine and Hygiene,* 49, 1, 53-62.

Mitchell, C.J., Monath, T.P., Sabattini, M.S., Cropp, C.B., Daffner, J.F., Calisher, C.H., Jakob, W.L. & Christensen, H.A. 1985, Arbovirus investigations in Argentina, 1977-1980. II.

Arthropod collections and virus isolations from Argentine mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 34, 5, 945-955.

Mohamed, M., McLees, A. & Elliott, R.M. 2009, Viruses in the Anopheles A, Anopheles B, and Tete serogroups in the Orthobunyavirus genus (family Bunyaviridae) do not encode an NSs protein, *Journal of virology,* 83, 15, 7612-7618.

Monath, T.P., Nuckolls, J.G., Berall, Bauer, H., Chappell, W.A. & Coleman, P.H. 1970, Studies on California encephalitis in Minnesota, *American Journal of Epidemiology,* 92, 1, 40-50.

Moussa, M.I., Imam, I.Z., Converse, J.D. & El-Karamany, R.M. 1974, Isolation of Matruh virus from Hyalomma marginatum ticks in Egypt, *The Journal of the Egyptian Public Health Association,* 49, 6, 341-348.

Mullen, G.L. & Durden, L. 2002, *Medical and Veterinary Entomology: Introduction,* Academic Press, Burlington, MA, USA.

Murphy, F.A., Harrison, A.K. & Whitfield, S.G. 1973, Bunyaviridae: morphologic and morphogenetic similarities of Bunyamwera serologic supergroup viruses and several other arthropod-borne viruses, *Intervirology,* 1, 4, 297-316.

Nashed, N.W., Olson, J.G. & el-Tigani, A. 1993, Isolation of Batai virus (Bunyaviridae:Bunyavirus) from the blood of suspected malaria patients in Sudan, *The American Journal of Tropical Medicine and Hygiene,* 48, 5, 676-681.

Niklasson, B., Espmark Aa., Jaenson, T.G.T. & Kjelsson, T. 1985, Inkoo virus isolated from Swedish mosquitoes for the first time, *Lakartidningen,* 82, 47, 4136.

Noda, Y., Uchinuno, Y., Shirakawa, H., Nagasue, S., Nagano, N., Ohe, R. & Narita, M. 1998, Aino Virus Antigen in Brain Lesions of a Naturally Aborted Bovine Fetus, *Veterinary pathology,* 35, 5, 409-411.

Ogg, M.M. & Patterson, J.L. 2007, RNA binding domain of Jamestown Canyon virus S segment RNAs, *Journal of virology,* 81, 24, 13754-13760.

Okuno, T. 1961, Immunological studies relating two recently isolated viruses, Germiston virus from South Africa and Ilesha virus from West Africa, to the Bunyamwera group, *The American Journal of Tropical Medicine and Hygiene,* 10, 223-226.

Ota, W.K., Watkins, H.M., Neri, P., Schmidt, M.L. & Schmidt, J.R. 1976, Arbovirus recoveries from mosquitoes collected in Gambela, Illubabor Province, Ethiopia, 1970, *Journal of medical entomology,* 13, 2, 173-178.

Oya, A., Okuno, T., Ogata, T., Kobayashii & Matsuyama, T. 1961, Akabane, a new arbor virus isolated in Japan, *Japanese journal of medical science & biology,* 14, 101-108.

Pachler, K., Ruzek, D. & Nowotny, N. 2013, Molecular characterization of the African orthobunyavirus Ilesha virus, *Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases,* 20, 124-130.

Pantuwatana, S., Thompson, W.H., Watts, D.M. & Hanson, R.P. 1972, Experimental infection of chipmunks and squirrels with La Crosse and Trivittatus viruses and

biological transmission of La Crosse virus by Aedes triseriatus, *The American Journal of Tropical Medicine and Hygiene,* 21, 4, 476-481.

Pappu, H.R., Jones, R.A. & Jain, R.K. 2009, Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead, *Virus research,* 141, 2, 219-236.

Parkin, W.E., Hammon, W.M. & Sather, G.E. 1972, Review of current epidemiological literature on viruses of the California arbovirus group, *The American Journal of Tropical Medicine and Hygiene,* 21, 6, 964-978.

Parsonson, I.M., Della-Porta, A.J. & Snowdon, W.A. 1981, Developmental disorders of the fetus in some arthropod-borne virus infections, *The American Journal of Tropical Medicine and Hygiene,* 30, 3, 660-673.

Parsonson, I.M., Della-Porta, A.J. & Snowdon, W.A. 1977, Congenital abnormalities in newborn lambs after infection of pregnant sheep with Akabane virus, *Infection and immunity,* 15, 1, 254-262.

Pastula, D.M., Hoang Johnson, D.K., White, J.L., Dupuis, A.P.,2nd, Fischer, M. & Staples, J.E. 2015, Jamestown Canyon Virus Disease in the United States-2000-2013, *The American Journal of Tropical Medicine and Hygiene,* 93, 2, 384-389.

Patterson, J.L., Holloway, B. & Kolakofsky, D. 1984, La Crosse virions contain a primer-stimulated RNA polymerase and a methylated cap-dependent endonuclease, *Journal of virology,* 52, 1, 215-222.

Pinheiro, F.P., Travassos da Rosa, A.P., Travassos da Rosa, J.F., Ishak, R., Freitas, R.B., Gomes, M.L., LeDuc, J.W. & Oliva, O.F. 1981, Oropouche virus. I. A review of clinical, epidemiological, and ecological findings, *The American Journal of Tropical Medicine and Hygiene,* 30, 1, 149-160.

Plassmeyer, M.L., Soldan, S.S., Stachelek, K.M., Roth, S.M., Martin-Garcia, J. & Gonzalez-Scarano, F. 2007, Mutagenesis of the La Crosse Virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide, *Virology,* 358, 2, 273-282.

Poglazov, A.B., Shliapnikova, O.V. & Prilipov, A.G. 2011, Characteristic of the new Khatanga virus genotype, *Vestnik Rossiiskoi akademii meditsinskikh nauk / Rossiiskaia akademiia meditsinskikh nauk,* (5), 5, 45-49.

Putkuri, N., Brummer-Korvenkontio, M., Vaheri, A. & Vapalahti, O. 2004, Inkoo-virus: hyttysten levittämä keskushermostoinfektion aiheuttaja Suomessa,, 21-22, 2247-2250.

Putkuri, N., Kurkela, S., Levanov, L., Huhtamo, E., Vaheri, A., Sironen, T. & Vapalahti, O. 2014, Isolation and characterization of a California encephalitis serogroup orthobunyavirus from Finnish mosquitoes, *Infection, Genetics and Evolution,* 22, 164-173.

Putkuri, N., Kantele, A., Levanov, L., Kivistö, I., Brummer-Korvenkontio, M., Vaheri, A. & Vapalahti, O. 2016, Acute Human Inkoo and Chatanga Virus Infections, Finland, *Emerging Infectious Diseases,* 22, 5

Rasmussen, L.D., Kristensen, B., Kirkeby, C., Rasmussen, T.B., Belsham, G.J., Bodker, R. & Botner, A. 2012, Culicoids as vectors of Schmallenberg virus, *Emerging infectious diseases,* 18, 7, 1204-1206.

Reese, S.M., Beaty, M.K., Gabitzsch, E.S., Blair, C.D. & Beaty, B.J. 2009, Aedes triseriatus Females Transovarially Infected With La Crosse Virus Mate More Efficiently Than Uninfected Mosquitoes, *Journal of medical entomology,* 46, 5, 1152 - 1158.

Reeves, W.C., Scrivani, R.P., Hardy, J.L., Roberts, D.R. & Nelson, R.L. 1970, Buttonwillow virus, a new Arbovirus isolated from mammals and Culicoides midges in Kern County, California, *The American Journal of Tropical Medicine and Hygiene,* 19, 3, 544-551.

Reguera, J., Malet, H., Weber, F. & Cusack, S. 2013, Structural basis for encapsidation of genomic RNA by La Crosse Orthobunyavirus nucleoprotein, *Proceedings of the National Academy of Sciences of the United States of America,* 110, 18, 7246-7251.

Reimann, C.A., Hayes, E.B., DiGuiseppi, C., Hoffman, R., Lehman, J.A., Lindsey, N.P., Campbell, G.L. & Fischer, M. 2008, Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007, *The American Journal of Tropical Medicine and Hygiene,* 79, 6, 974-979.

Reisen, W.K. 2002, *Medical and Veterinary Entomology: Epidemiology of vector-borne diseases,* Academic Press, Burlington, MA, USA.

Ritter, D.G. & Feltz, E.T. 1974, On the natural occurrence of California encephalitis virus and other arboviruses in Alaska, *Canadian journal of microbiology,* 20, 10, 1359-1366.

Roca-Garcia, M. 1944, The Isolation of Three Neurotropic Viruses from Forest Mosquitoes in Eastern Colombia, *Journal of Infectious Diseases,* 75, 2, 160-169.

Rodrigues, F.M., Singh, P.B., Dandawate, C.N., Soman, R.S. & Bhatt, P.N. 1977, Kaikalur virus--a new arthropod-borne virus belonging to the Simbu group isolated in India from Culex tritaeniorhynchus (Giles), *The Indian journal of medical research,* 66, 5, 719-725.

Rogstad, D.K., Schiffman, E., Neitzel, D. & Baddour, L.M. 2015, Severe Sepsis Caused by California Serogroup Orthobunyavirus, *Emerging infectious diseases,* 21, 10, 1876-1877.

Russell, R.C., Cloonan, M.J., Wells, P.J. & Vale, T.G. 1991, Mosquito (Diptera: Culicidae) and arbovirus activity on the south coast of New South Wales, Australia, in 1985-1988, *Journal of medical entomology,* 28, 6, 796-804.

Sabattini, M.S., Shope, R.E. & Vanella, J.M. 1965, Serological survey for arboviruses in Cordoba Province, Argentina, *The American Journal of Tropical Medicine and Hygiene,* 14, 6, 1073-1078.

Sahu, S.P., Landgraf, J., Wineland, N., Pedersen, D., Alstad, D. & Gustafson, G. 2000, Isolation of Jamestown Canyon virus (California virus group) from vesicular lesions of

a horse, *Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc,* 12, 1, 80-83.

Sailleau, C., Boogaerts, C., Meyrueix, A., Laloy, E., Breard, E., Viarouge, C., Desprat, A., Vitour, D., Doceul, V., Boucher, C., Zientara, S., Nicolier, A. & Grandjean, D. 2013, Schmallenberg virus infection in dogs, France, 2012, *Emerging infectious diseases,* 19, 11, 1896-1898.

Saluzzo, J., Germain, M., Huard, M., Robin, Y., Gonzalez, J., Herve, J., Georges, A., Heme, G. & Digoutte, J. 1983, Le virus bozo (ArB 7343): Un nouvel arbovirus du groupe bunyamwera isolé en république centrafricaine; sa transmission expérimentale par Aedes aegypti, *Annales de l'Institut Pasteur / Virologie,* 134, 2, 221-232.

Sanchez-Vargas, I., Travanty, E.A., Keene, K.M., Franz, A.W., Beaty, B.J., Blair, C.D. & Olson, K.E. 2004, RNA interference, arthropod-borne viruses, and mosquitoes, *Virus research,* 102, 1, 65-74.

Sather, G.E. & Hammon, W.M. 1967, Antigenic patterns within the California-encephalitis-virus group, *The American Journal of Tropical Medicine and Hygiene,* 16, 4, 548-557.

Savji, N., Palacios, G., Travassos da Rosa, A., Hutchison, S., Celone, C., Hui, J., Briese, T., Calisher, C.H., Tesh, R.B. & Lipkin, W.I. 2011, Genomic and phylogenetic characterization of Leanyer virus, a novel orthobunyavirus isolated in northern Australia, *Journal of General Virology,* 92, 7, 1676-1687.

Schaffer, P.A. & Scherer, W.F. 1972, Growth of a candidate arbovirus (Tsuruse) in Aedes aegypti mosquitoes following intrathoracic inoculation, *Proceedings of the Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.),* 139, 4, 1298-1304.

Scherer, W.F., Anderson, K., Dickerman, R.W. & Ordonez, J.V. 1972, Studies of Patois group arboviruses in Mexico, Guatemala, Honduras, and British Honduras, *The American Journal of Tropical Medicine and Hygiene,* 21, 2, 194-200.

Scherer, W.F., Campillo-Sainz, C., Dickerman, R.W., Diaz-Najera, A. & Madalengoitia, J. 1967, Isolation of Tlacotalpan virus, a new Bunyamwera-group virus from Mexican mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 16, 1, 79-91.

Scherer, W.F., Dickerman, R.W., Ordonez, J.V., Seymour, C.,3rd, Kramer, L.D., Jahrling, P.B. & Powers, C.D. 1976, Ecologic studies of Venezuelan encephalitis virus and isolations of Nepuyo and Patois viruses during 1968-1973 at a marsh habitat near the epicenter of the 1969 outbreak in Guatemala, *The American Journal of Tropical Medicine and Hygiene,* 25, 1, 151-162.

Schmaljohn, C.S. & Hooper, J.W. 2007, *Bunyaviridae: the viruses and their replication.In Fields' virology,* Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia, Pa.

Service, M.W. 2008, Culicine mosquitoes, *Medical Entomology for Students,* 53.

Sexton, D.J., Rollin, P.E., Breitschwerdt, E.B., Corey, G.R., Myers, S.A., Dumais, M.R., Bowen, M.D., Goldsmith, C.S., Zaki, S.R., Nichol, S.T., Peters, C.J. & Ksiazek, T.G. 1997, Life-threatening Cache Valley virus infection, *The New England journal of medicine,* 336, 8, 547-549.

Seymour, C., Peralta, P.H. & Montgomery, G.G. 1983, Viruses isolated from Panamanian sloths, *The American Journal of Tropical Medicine and Hygiene,* 32, 6, 1435-1444.

Shope, R.E., Causey, C.E. & Causey, O.R. 1961, Itaqui Virus, a New Member of Arthropod-Borne Group C, *The American Journal of Tropical Medicine and Hygiene,* 10, 2, 264-265.

Simkova, A. & Sluka, F. 1973, Isolation of Tahyna virus from the blood of a case of influenza-like disease, *Acta virologica,* 17, 1, 94.

Sirhongse, S. & Johnson, C.M. 1965, Wyeomyia Subgroup of Arbovirus: Isolation from Man, *Science (New York, N.Y.),* 149, 3686, 863-864.

Sluka, F. 1969, *Arboviruses of the California Complex and the Bunyamwera Group: The Clinical Picture of the Tahyna Virus Infection.* Proceedings of the Symposium held at Smolenice near Bratislava October 18-21, 1966.

Smithburn, K.C., Haddow, A.J. & Mahaffy, A.F. 1946, A neurotropic virus isolated from Aedes mosquitoes caught in the Semliki forest, *The American Journal of Tropical Medicine and Hygiene,* 26, 189-208.

Smithburn, K.C., Kokernot, R.H., Heymann, C.S., Weinbren, M.P. & Zentkowsky, D. 1959, Neutralizing antibodies for certain viruses in the sera of human beings residing in Northern Natal, *South African medical journal,* 33, 27, 555-561.

Smithburn, K.C., Mahaffy, A.F. & Paul, J.H. 1941, Bwamba Fever and Its Causative Virus, *The American Journal of Tropical Medicine and Hygiene,* 1-21, 1, 75-90.

Soldan, S.S., Hollidge, B.S., Wagner, V., Weber, F. & Gonzalez-Scarano, F. 2010, La Crosse virus (LACV) Gc fusion peptide mutants have impaired growth and fusion phenotypes, but remain neurotoxic, *Virology,* 404, 2, 139-147.

Spence, L., Anderson, C.R., Aitken, T.H. & Downs, W.G. 1967, Bushbush, Ieri and Lukuni viruses, three unrelated new agents isolated from Trinidadian forest mosquitoes, *Proceedings of the Society for Experimental Biology and Medicine.Society for Experimental Biology and Medicine (New York, N.Y.),* 125, 1, 45-50.

Spence, L., Anderson, C.R., Aitken, T.H. & Downs, W.G. 1966, Nepuyo virus, a new group C agent isolated in Trinidad and Brazil. I. Isolation and properties of the Trinidadian strain, *The American Journal of Tropical Medicine and Hygiene,* 15, 1, 71-74.

Spence, L., Anderson, C.R., Aitken, T.H. & Downs, W.G. 1962a, Bimiti virus, a new agent isolated from Trinidadian mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 11, 414-417.

Spence, L., Anderson, C.R., Aitken, T.H. & Downs, W.G. 1962b, Melao virus, a new agent isolated from Trinidadian mosquitoes, *The American Journal of Tropical Medicine and Hygiene,* 11, 687-690.

Spence, L. & Downs, W.G. 1968, Virological investigations in Guyana, 1956-1966, *The West Indian medical journal,* 17, 2, 83-89.

Srihongse, S., Galindo, P. & Grayson, M.A. 1966, Isolation of group C arboviruses in Panama including two new members, Patois and Zegla, *The American Journal of Tropical Medicine and Hygiene,* 15, 3, 379-384.

Srihongse, S., Grayson, M.A. & Deibel, R. 1984, California serogroup viruses in New York State: the role of subtypes in human infections, *The American Journal of Tropical Medicine and Hygiene,* 33, 6, 1218-1227.

St George, T.D., Cybinski, D.H., Filippich, C. & Carley, J.G. 1979, The isolation of three Simbu group viruses new to Australia, *The Australian Journal of Experimental Biology and Medical Science,* 57, 6, 581-582.

St George, T.D., Standfast, H.A., Cybinski, D.H., Filippich, C. & Carley, J.G. 1980, Peaton virus: a new Simbu group arbovirus isolated from cattle and Culicoides brevitarsis in Australia, *Australian Journal of Biological Sciences,* 33, 2, 235-243.

Standfast, H.A., Dyce, A.L., St George, T.D., Muller, M.J., Doherty, R.L., Carley, J.G. & Filippich, C. 1984, Isolation of arboviruses from insects collected at Beatrice Hill, Northern Territory of Australia, 1974-1976, *Australian Journal of Biological Sciences,* 37, 5-6, 351-366.

Stevenson, A.E. & Holmes, I.H. 1972, Electron microscopy of Koongol group arboviruses, *Australian Journal of Biological Sciences,* 25, 1, 53-60.

Sudia, W.D., Fernandez, L., Newhouse, V.F., Sanz, R. & Calisher, C.H. 1975, Arbovirus vector ecology studies in Mexico during the 1972 Venezuelan equine encephalitis outbreak, *American Journal of Epidemiology,* 101, 1, 51-58.

Sudia, W., Chamberl, RW, Calisher, C. & Newhouse, V. 1971, California Group Arboviruses - Isolations from Mosquitos in North-America, *Mosquito News,* 31, 4, 576-&.

Tao, H., Xia, S.M., Chan, Z.Y., Song, G. & Yanagihara, R. 1987, Morphology and morphogenesis of viruses of hemorrhagic fever with renal syndrome. II. Inclusion bodies--ultrastructural markers of hantavirus-infected cells, *Intervirology,* 27, 1, 45-52.

Taylor, D.J., Lewis, A.L., Edman, J.D. & Jennings, W.L. 1971, California group arboviruses in Florida. Host-vector relations, *The American Journal of Tropical Medicine and Hygiene,* 20, 1, 139-145.

Taylor, K.G. & Peterson, K.E. 2014, Innate immune response to La Crosse virus infection, *Journal of neurovirology,* 20, 2, 150-156.

Tesh, R.B. & Beaty, B.J. 1983, Localization of California serogroup viruses in mosquitoes, *Progress in clinical and biological research,* 123, 67-75.

Thompson, W.H. 1983, Vector-virus relationships, *Progress in clinical and biological research,* 123, 57-66.

Thompson, W.H., Anslow, R.O., Hanson, R.P. & Defoliart, G.R. 1972, La Crosse virus isolations from mosquitoes in Wisconsin, 1964-68, *The American Journal of Tropical Medicine and Hygiene,* 21, 2, 90-96.

Thompson, W.H. & Beaty, B.J. 1977, Venereal transmission of La Crosse (California encephalitis) arbovirus in Aedes triseriatus mosquitoes, *Science (New York, N.Y.),* 196, 4289, 530-531.

Thompson, W.H., Kalfayan, B. & Anslow, R.O. 1965, Isolation of California Encephalitis Group Virus from a Fatal Human Illness, *American Journal of Epidemiology,* 81, 245-253.

Tignor, G.H., Burrage, T.G., Smith, A.L., Shope, R.E. & Bishop, D.H. 1983, California serogroup gene structure-function relationships: virulence and tissue tropisms, *Progress in clinical and biological research,* 123, 129-138.

Tikasingh, E.S., Ardoin, P. & Williams, M.C. 1974, First isolation of Catu virus from a human in Trinidad, *Tropical and geographical medicine,* 26, 4, 414-416.

Tingström, O., Wesula, L.O., Näslund, J., Spyckerelle, I., Engdahl, C., Von Schoenberg, P., Ahlm, C., Evander, M. & Bucht, G. 2016, Detection of Sindbis and Inkoo Virus RNA in Genetically Typed Mosquito Larvae Sampled in Northern Sweden, *Vector Borne Zoonotic Disease*, 16 (7), 461-467.

Top, F.H.,Jr, Kraivapan, C., Grossman, R.A., Rozmiarek, H., Edelman, R. & Gould, D.J. 1974, Ingwavuma virus in Thailand. Infection of domestic pigs, *The American Journal of Tropical Medicine and Hygiene,* 23, 2, 251-257.

Traavik, T., Mehl, R. & Wiger, R. 1985, Mosquito-borne arboviruses in Norway: further isolations and detection of antibodies to California encephalitis viruses in human, sheep and wildlife sera, *The Journal of hygiene,* 94, 1, 111-122.

Traavik, T., Mehl, R. & Wiger, R. 1978, California encephalitis group viruses isolated from mosquitoes collected in Southern and Arctic Norway, *Acta Pathologica et Microbiologica Scandinavica. Section B, Microbiology,* 86B, 6, 335-341.

Ushijima, H., Klimas, R., Kim, S., Cash, P. & Bishop, D.H. 1980, Characterization of the viral ribonucleic acids and structural polypeptides of Anopheles A, Bunyamwera, Group C, California, Capim, Guama, Patois, and Simbu bunyaviruses, *The American Journal of Tropical Medicine and Hygiene,* 29, 6, 1441-1452.

Vaheri, A., Henttonen, H., Voutilainen, L., Mustonen, J., Sironen, T. & Vapalahti, O. 2013, Hantavirus infections in Europe and their impact on public health, *Reviews in medical virology,* 23, 1, 35-49.

van Eeden, C., Williams, J.H., Gerdes, T.G., van Wilpe, E., Viljoen, A., Swanepoel, R. & Venter, M. 2012, Shuni virus as cause of neurologic disease in horses, *Emerging infectious diseases,* 18, 2, 318-321.

Vanlandingham, D.L., Davis, B.S., Lvov, D.K., Samokhvalov, E., Lvov, S.D., Black, W.C., Higgs, S. & Beaty, B.J. 2002, Molecular characterization of California serogroup

viruses isolated in Russia, *The American Journal of Tropical Medicine and Hygiene,* 67, 3, 306-309.

Vapalahti, O., Kallio-Kokko, H., Narvanen, A., Julkunen, I., Lundkvist, A., Plyusnin, A., Lehvaslaiho, H., Brummer-Korvenkontio, M., Vaheri, A. & Lankinen, H. 1995, Human B-cell epitopes of Puumala virus nucleocapsid protein, the major antigen in early serological response, *Journal of medical virology,* 46, 4, 293-303.

Vapalahti, O., Plyusnin, A., Cheng, Y., Manni, T., Brummer-Korvenkontio, M. & Vaheri, A. 1996, Inkoo and Tahyna, the European California serogroup bunyaviruses: sequence and phylogeny of the S RNA segment, *The Journal of general virology,* 77 (Pt 8), Pt 8, 1769-1774.

Walder, R., Suarez, O.M. & Calisher, C.H. 1984, Arbovirus studies in southwestern Venezuela during 1973-1981. II. Isolations and further studies of Venezuelan and eastern equine encephalitis, Una, Itaqui, and Moju viruses, *The American Journal of Tropical Medicine and Hygiene,* 33, 3, 483-491.

Watts, D.M., DeFoliart, G.R. & Yuill, T.M. 1976, Experimental transmission of trivittatus virus (California virus group) by Aedes trivittatus, *The American Journal of Tropical Medicine and Hygiene,* 25, 1, 173-176.

Watts, D.M., Pantuwatana, S., DeFoliart, G.R., Yuill, T.M. & Thompson, W.H. 1973, Transovarial Transmission of LaCrosse Virus (California Encephalitis Group) in the Mosquito, Aedes triseriatus, *Science,* 182, 4117, 1140 -1141.

Watts, D.M., Thompson, W.H., Yuill, T.M., DeFoliart, G.R. & Hanson, R.P. 1974, Overwintering of La Crosse virus in Aedes triseriatus, *The American Journal of Tropical Medicine and Hygiene,* 23, 4, 694-700.

Weaver, S.C., Coffey, L.L., Nussenzveig, R., Ortiz, D. & Smith, D. 2004, *Microbe-Vector Interactions in Vector-Borne Diseases: Vector competence,* Cambridge University Press, West Nyack, NY, USA.

Whitehouse, C.A. 2004, Crimean-Congo hemorrhagic fever, *Antiviral Research,* 64, 3, 145-160.

Whitman, L. & Shope, R.E. 1962, The California complex of arthropod-borne viruses and its relationship to the Bunyamwera group through Guaroa virus, *The American Journal of Tropical Medicine and Hygiene,* 11, 691-696.

Williams, J.E., Imlarp, S., Top, F.H.,Jr, Cavanaugh, D.C. & Russell, P.K. 1976, Kaeng Khoi virus from naturally infected bedbugs (cimicidae) and immature free-tailed bats, *Bulletin of the World Health Organization,* 53, 4, 365-369.

Williams, M.C., Woodall, J.P. & Corbet, P.S. 1965, Nyando Virus: a Hitherto Undescribed Virus Isolated from Anopheles Funestus Giles Collected in Kenya, *Archiv fur die Gesamte Virusforschung,* 15, 422-427.

Wills, W., Pidcoe, V., Carroll, D.F. & Satz, J.E. 1974, *Isolation of California group arboviruses from Pennsylvania mosquitoes--1971, 1972.*

Woodring, J., Chandler, L.J., Oray, C.T., McGaw, M.M., Blair, C.D. & Beaty, B.J. 1998, Short report: Diapause, transovarial transmission, and filial infection rates in geographic strains of La Crosse virus-infected Aedes triseriatus, *The American Journal of Tropical Medicine and Hygiene,* 58, 5, 587-588.

Yanase, T., Fukutomi, T., Yoshida, K., Kato, T., Ohashi, S., Yamakawa, M. & Tsuda, T. 2004, The emergence in Japan of Sathuperi virus, a tropical Simbu serogroup virus of the genus Orthobunyavirus, *Archives of Virology,* 149, 5, 1007-1013.

Yanase, T., Kato, T., Aizawa, M., Shuto, Y., Shirafuji, H., Yamakawa, M. & Tsuda, T. 2012, Genetic reassortment between Sathuperi and Shamonda viruses of the genus Orthobunyavirus in nature: implications for their genetic relationship to Schmallenberg virus, *Archives of Virology,* 157, 8, 1611-1616.

Yanase, T., Maeda, K., Kato, T., Nyuta, S., Kamata, H., Yamakawa, M. & Tsuda, T. 2005, The resurgence of Shamonda virus, an African Simbu group virus of the genus Orthobunyavirus, in Japan, *Archives of Virology,* 150, 2, 361-369.

Yang, D.K., Kim, B.H., Kweon, C.H., Nah, J.J., Kim, H.J., Lee, K.W., Yang, Y.J. & Mun, K.W. 2008, Serosurveillance for Japanese encephalitis, Akabane, and Aino viruses for Thoroughbred horses in Korea, *Journal of veterinary science,* 9, 4, 381-385.

Yuill, T.M. 1983, The role of mammals in the maintenance and dissemination of La Crosse virus, *Progress in clinical and biological research,* 123, 77-87.

Zamparo, J.M., Andreadis, T.G., Shope, R.E. & Tirrell, S.J. 1997, Serologic evidence of Jamestown Canyon virus infection in white-tailed deer populations from Connecticut, *Journal of wildlife diseases,* 33, 3, 623-627.

Zarnke, R.L., Calisher, C.H. & Kerschner, J. 1983, SEROLOGIC EVIDENCE OF ARBOVIRUS INFECTIONS IN HUMANS AND WILD ANIMALS IN ALASKA, *Journal of wildlife diseases,* 19, 3, 175-179.

Zeller, H.G., Diallo, M., Angel, G., Traore-Lamizana, M., Thonnon, J., Digoutte, J.P. & Fontenille, D. 1996, Ngari virus (Bunyaviridae: Bunyavirus). First isolation from humans in Senegal, new mosquito vectors, its epidemiology, *Bulletin de la Societe de pathologie exotique (1990),* 89, 1, 12-16.

Zhang, H.L., Zhang, Y.Z., Yang, W.H., Feng, Y., Nasci, R.S., Yang, J., Liu, Y.H., Dong, C.L., Li, S., Zhang, B.S., Yin, Z.L., Wang, P.Y., Fu, S.H., Li, M.H., Liu, F., Zhang, J., Sun, J., Li, C.W., Gao, X.Y., Liu, H., Wang, H.Y., Petersen, L.R. & Liang, G.D. 2013, Mosquitoes of Western Yunnan Province, China: seasonal abundance, diversity, and arbovirus associations, *PloS one,* 8, 10, e77017.

Zhang, Z. 2011, Animal biodiversity: An introduction to higher-level classification and taxonomic richness, *Zootaxa,* 3148, 1-237.

APPENDIX 1.

Summary of the serogroups in genus orthobunyavirus. The table shows the species that belong to each serogroup, the isolates of every species, their disease association, their distribution and arthropod vectors from which they have been isolated.

Serogroup	Species	Strains/types	Disease: Isolation / antibodies detected	Isolation area/ Distribution	*Arthropod vector (isolated from)*
Anopheles A (Calisher et al. 1980)	*Anopheles A virus*	Anopheles A virus (ANAV)	- (Roca-Garcia 1944)	Columbia	*Anopheles (Kerteszia) boliviensis*
		Las Maloyas virus (LMV)	- (Mitchell et al. 1985)	Argentina	*Anopheles albitarsis*
		Lukuni virus (LUKV)	- (Spence et al. 1967)	Trinidad	*Ochlerotatus scapularis*
		Trombetas virus (TRMV)	- (Calisher et al. 1973)		
	Tacaiuma virus	Tacaiuma virus (TCMV)	- Human (Calisher et al. 1980, Causey et al. 1961)	Brazil	*Anopheles sp.*
		CoAr1071 virus (CA1071 V)	- (Calisher et al. 1980)	Columbia	*Wyeomyia sp.*
		CoAr3624 virus (CA3624 V)	- (Calisher et al. 1980, Calisher et al. 1973)	Columbia	*Anopheles neiuai*
		Virgin River virus (VRV)	- (Calisher et al. 1980)	Arizona, USA	*Anopheles freebonti*
Anopheles B	*Anopheles B virus*	Anopheles B virus (ANBV)	- (Roca-Garcia 1944)	Colombia	*Anopheles (Kerteszia) boliviensis*
		Boraceia virus (BORV)	- (Lopes Ode et al. 1966)	Brasilia	
Bakau	*Bakau virus*	Bakau virus (BAKV)	Macaca fascicularis, bats, wild birds, fowl (Marchette 1994)	Malaysia/Pakistan	*Culex sp.*
		Ketapang virus (KETV)	- (Marchette 1994)	Malaysia	*Culex (Lophoceratomyia) sp.*
		Nola virus (NOLAV)	- (Marchette 1994)	Central Africa	*Culex perfuscus*
		Tanjong Rabok virus (TRV)	M. nemestrina, humans, squirrel, bats, wild birds (Marchette 1994)	Malaysia	Not isolated from arthropods
		Telok Forest virus (TFV)	M. nemestrina (Marchette 1994)	Malaysia	Not isolated from arthropods

Bunyamwera	*Bunyamwera virus*	Bunyamwera virus (BUNV)	Humans, monkeys, domestic animals, rodents, birds (Kokernot et al. 1958, Smithburn, Haddow & Mahaffy 1946)	Africa	*Aedes spp., Mansonia sp., Culex sp.*
		Batai virus (BATV)	Cattle, humans (Nashed, Olson & el-Tigani 1993, Liu et al. 2014, Hubalek 2008)	Europe, Asia, Sudan	*Culex gelidus, An. maculipennis*
		Cache Valley virus (CVV)	Cattle, humans, horses, deer, rodents (Chung et al. 1990, Sexton et al. 1997, Holden, Hess 1959, Buescher et al. 1970, McLean, Calisher & Parham 1987)	North and Central America	*Mammalphilic mosquito* (Andreadis et al. 2014)
		Fort Sherman virus (FSV)	Humans (Mangiafico et al. 1988)	Panama	Not isolated from arthropods
		Germiston virus (GERV)	Humans, rodents (Kokernot et al. 1960, Henderson et al. 1972)	Africa	*Culex Theileri, Culex Rubinotus*
		Ilesha virus (ILEV) reassortant	Humans (Pachler, Ruzek & Nowotny 2013, Okuno 1961)	Africa	*Anopheles gambiae*
		Ngari virus (NRIG) reassortant (BUNMV+BATV)	Humans (Gerrard et al. 2004, Zeller et al. 1996)	East, sub-Sahara Africa	*Stegomyia simpsoni*
		Northway virus (NORV)	Humans, horses, deer (Zarnke, Calisher & Kerschner 1983, Campbell et al. 1991)	North America	*Anopheles freeborni, Aedes sierrensis*
		Shokwe virus (SHOV)	Humans (Hunt, Calisher 1979) ArboCat*	South Africa, Senegal, Kenya, Ivory Coast	*Aedes, Anopheles and Mansonia mosquitoes*
		Xingu virus (XINV) BeH 388464 *strain*	Humans (Marchette 1994, Calisher et al. 1988)	Brazil	Not isolated from arthropods
		Birao virus (BIRV) DakAr B 2198	- ArboCat*	Central Africa	*Anopheles pharoensis, Anopheles squamosus*
		Bozo virus (BOZOV)	Humans (Saluzzo et al. 1983)	Central Africa	*Stegomyia opok, A. africanus*
		Lokern virus (LOKV)	Humans, rodents, hares, domestic	USA	*Culex tarsalis, culicoides*

			animals (Anonymous1970c, Calisher et al. 1986)		
		Maguari virus (MAGV)CACV BeAr7272	Horses (Causey et al. 1961, Spence, Downs 1968, Sabattini, Shope & Vanella 1965) human, cattle	South America	*Mammalphilic mosquito* (Calisher et al. 1983)
		Mboke virus (MBOV)†	- ArboCat*	Cameroon	*Och. ingrami*
		Playas virus (PLAV)	- (Calisher et al. 1983)	Ecuador	*Aedes taeniorhynchus*
		Potosi virus (POTV) reassortant (CCV+KRIV)	Deer (Francy et al. 1990, McLean et al. 1996)	USA	*Stegomyia albopicta, Mammalphilic mosquito* (Anderson et al. 2015)
		Santa Rosa virus (SARV)	- (Calisher et al. 1986, Sudia et al. 1975)	Mexico	*Aedes angustivittatus*
		Tensaw virus (TENV)	Humans, dogs, cows (Coleman 1969, Chamberlain, Sudia & Coleman 1969)	USA	*Anopheles sp., Psorophora sp., Aedes sp., Culex sp., Mansonia sp.*
		Tlacotalpan virus (TLAV)	Humans, cattle, pigs, chickens (Scherer et al. 1967)	Mexico	*Maneonia titillans, Anopheles albimanus, Aedes taeniorhynchus,*
		Anhembi virus (AMBV)	Rodent, humans (DE Souza Lopes et al. 1975)	Brazil	*Phoniomyia pilicauda, Trichoprosopon pallidiventer*
		Cholul virus (CHLV) reassortant (CVV+POTV)	Humans, horses, sheep (Blitvich et al. 2012a, Blitvich et al. 2012b)	Mexico	Mosquitoes
	Guaroa virus	Guaroa virus (GROV)	Humans (Groot et al. 1959)	South America	*Anopheles sp.*
	Kairi virus	Kairi virus (KRIV)	Horses, humans, donkeys (Calisher et al. 1988, Anderson et al. 1960)	South America	*Ochlerotatus scapularis, Wyeomyia sp., Psorophora sp. and Culex spissipes*
	Main Drain virus	Main Drain virus (MDV) reassortant (CCV+KRIV)	Horses, Lepus Californicus, ruminants (Emmons et al. 1983)	USA	*Culicoides variipennis, Ochlerotatus taeniorhynchus*

Bwamba (Groseth et al. 2014)	*Bwamba virus*	Bwamba virus (BWAV)	Humans, donkeys, birds, monkeys (Smithburn, Mahaffy & Paul 1941, Lutwama et al. 2002)	Africa	*Anopheles funestus, Anopheles gambiae*
		Pongola virus (PGAV)	Humans, ruminants (Kokernot et al. 1957, Kalunda et al. 1985)	Africa	*Neomelaniconion circumluteolus, other Aedes sp., Mansonia sp., Anopheles sp.*
California (Calisher 1983)	*California encephalitis virus*	California encephalitis virus (CEV)	Humans, horses, cows, rabbits, ground squirrels (Hammon, Reeves & Sather 1952, Hammon, Reeves 1952)	USA, Canada	*Aedes dorsalis, Culex tarsalis, other Aedes sp., Culiseta sp.*
		Inkoo virus (INKV)	Humans, cows, deer, hares (Brummer-Korvenkontio et al. 1973, Brummer-Korvenkontio 1973a)	Northern Europe	*Ochlerotatus communis/punctor*
		Jamestown Canyon virus (JCV)	Humans, deer, horses, moose (DeFoliart et al. 1969, Zamparo et al. 1997, Grimstad et al. 1986, Sahu et al. 2000, McFarlane et al. 1981a)	USA, Canada	*Aedes sp., Culiseta sp.,* horsefly and deerfly
		La Crosse virus (LACV)	Humans, chipmunks, squirrels (Thompson, Kalfayan & Anslow 1965, Balfour et al. 1973, Gauld et al. 1975, Ksiazek, Yuill 1977)	USA	*Ochlerotatus triseriatus, other Aedes sp. and Culex sp.*
		Snowshoe hare virus (SSHV)	Lepus americanus, humans, horses, moose, deer (Burgdorfer, Newhouse & Thomas 1961, Heath et al. 1989, Meier-Stephenson et al. 2007, McLean 1983, McFarlane et al. 1981b)	North America, Russia	*Aedes sp. and Culiseta sp.*
		South river virus (SORV)	Horses, sheep, dogs (Sudia et al. 1971, Blitvich et al. 2012d, Blitvich et al. 2012c)	South America, North America	*Aedes sp., Anopheles sp., Culex sp. and Culiseta sp.*

		Tahyna virus (TAHV)	Human "Valtice fever", hares, hedgehogs, rodents, cattle, horses, wild birds (Bardos et al. 1975, Hubalek 2008, Lu et al. 2009)	Europe, Africa, Asia	*Aedes sp., Ochlerotatus sp. and Culiseta sp., Culex sp., Anopheles sp.*
		Keystone virus (KEYV)	Horses, humans, rabbits, rats (Bond et al. 1966, Parkin, Hammon & Sather 1972)	USA	*Ochlerotatus sp., Culex, sp, Anopheles sp.*
		Melao virus (MELV)	Humans, horses (Spence et al. 1962b, Calisher et al. 1987)	Trinidad, Brazil, Argentina	*Aedes sp. and Psorophora sp*
		San Angelo virus (SAV)	Deer (Sudia et al. 1971, Sather, Hammon 1967)	USA	*Anopheles pseudopunctipennis*
		Serra do Navio virus (SDNV)	Humans, large American opossums, Proechimys (South American spiny rats) (LeDuc 1987, Karabatsos 1985)	Brazil	*Ochlerotatus fulvus*
		Trivittatus virus (TVTV)	Humans (Sather, Hammon 1967, Lewis et al. 1965)	USA	*Ochlerotatus trivittatus and other Aedes sp., Culex sp. and Mansonia sp.*
		Chatanga virus (CHATV)	Human (Lavrent'ev et al. 2008, Putkuri et al. 2014)	Russia, Finland	*Ochlerotatus sp., Aedes sp.*
Capim (Ushijima et al. 1980)	*Acara virus*	Acara virus (ACAV)	- (Calisher et al. 1983)	Panama, Brazil	*Culex sp.*
		Moriche virus (MORV)	- (Jonkers et al. 1968a)	Trinidad	*Culex amazonensis*
	Benevides virus	Benevides virus (BVSV)	- ArboCat*	Brazil	*Culex sp.*
	Bushbush virus	Bushbush virus (BSBV)	- (Spence et al. 1967, Jonkers et al. 1968a)	Brazil, Trinidad	*Culex amazonensis, Cx. accelerans*
		Benfica virus (BENV)	Rodents, ArboCat*	Brazil	*Culex sp.*
		Juan Diaz virus (JDV)	- (Anonymous1970b)	Panama	*Not isolated from arthropods*
	Guajara virus	Guajara virus (GJAV)	- (Calisher et al. 1983)	Panama	*Culex sp.*
	Capim virus	Capim virus (CAPV)	Rodents, ArboCat*	Brazil	*Culex sp.*

Group C (Hang et al. 2014)	*Caraparu virus*	Caraparu virus (CAPV)	Humans, rodents (Causey et al. 1961, Iversson et al. 1987)	South America	*Culex sp., Wyeomyia sp., Aedes sp.*
		Apeu virus (APEUV)	Humans (Causey et al. 1961, Gibbs, Bruckner & Schenker 1964)	Brazil	*Aedes sp., Culex sp.*
		Ossa virus (OSSAV)	Humans (Derodaniche, Paesdeandrade & Galindo 1964)	Panama	*Culex sp.*
		Bruconha virus (BRUV)	- (Calisher et al. 1983)	Brazil	*Culex sp.*
		Vinces virus (VINV)	- (Calisher et al. 1983)	Ecuador	*Culex vomerifer*
		Itaya virus reassortant	Humans (Hontz et al. 2015)	Peru	Not isolated from arthropods
	Madrid virus	Madrid virus (MADV)	Humans (Derodaniche, Paesdeandrade & Galindo 1964)	Panama	*Culex sp.*
	Marituba virus	Marituba virus (MTBV)	Humans (Causey et al. 1961)	Brazil	*Culex sp.*
		Murutucu virus (MURV)	Humans, rodents (Causey et al. 1961)	Brazil	*Sabethine sp.*
		Nepuyo virus (NEPV)	Humans, rodents, bats, horses (Calisher et al. 1971, Spence et al. 1966, Scherer et al. 1976)	Brazil, Trinidad, Hondurans, Mexico, Panama	*Culex sp.*
		Restan virus (RESV)	Humans, horses, bats, birds (Calisher et al. 1971, Jonkers et al. 1967)	Trinidad, Surinam, Hondurans	*Culex portesi*
		Gumbo Limbo virus (GLV)	Cotton rats, horses (Calisher et al. 1971, Henderson et al. 1969)	South Florida, Hondurans	*Culex sp. Aedes taeniorhynchus*
	Oriboca virus	Oriboca virus (ORIV)	Humans, rodents (Metselaar 1966, Causey et al. 1961)	Surinam, Brazil	*Mansonia sp., Psorophora sp. Culex sp. Aedes sp. Sabethini sp.*
		Itaqui virus (ITQV)	Humans, rodents (Shope, Causey & Causey 1961, Walder, Suarez & Calisher 1984)	Brazil, Venezuela	*Culex vomerifer, other Culex sp. Psorophora sp.*

Gamboa (Calisher et al. 1981)	*Gamboa virus*	Gamboa virus (GAMV)	- (Calisher et al. 1981, Calisher et al. 1983)	Panama	*Aedeomyia squamipennis*
		Pueblo Viejo virus (PVV)	- (Calisher et al. 1981, Calisher et al. 1983)	Ecuador	*Aedeomyia squamipennis*
	Alajuela virus	Alajuela virus (ALJV)	- (Calisher et al. 1981)	Panama	*Aedeomyia squamipennis*
		San Juan virus (SJV)	Wild and domestic birds (Calisher et al. 1981, Calisher et al. 1983)	Ecuador, Argentina	*Aedeomyia squamipennis*
Guama (Ushijima et al. 1980)	*Bertioga virus*	Bertioga virus (BERV)	-(De Souza Lopes et al. 1975)	Brazil	Not isolated from arthropods
		Cananeia virus (CNAV)	-(Calisher et al. 1983)	Brazil	*Culex sacchette*
		Guaratuba virus (GTBV)	Birds (Calisher et al. 1983)	Brazil	*Och. serratus, anopheles cruzii*
		Itimirim virus (ITIV)	Rodents (Calisher et al. 1983)	Brazil	Not isolated from arthropods
		Mirim virus (MIRV)	Rodents (Calisher et al. 1983)	Brazil	*Culex sp., Aedes sp. Pso. ferox*
	Bimiti virus	Bimiti virus (MIMV)	Rodents, humans (Spence et al. 1962a)	Trinidad, Brazil, French Guiana, Surinam	*Culex species*
	Catu virus	Catu virus (CATUV)	Humans, rodents, bats (Causey et al. 1961, Tikasingh, Ardoin & Williams 1974, Jonkers et al. 1968b) ArboCat*	Brazil, Trinidad, French Guiana	*Culex portesi, other Culex sp., Anopheles sp.*
	Guama virus	Guama virus (GMAV)	Humans, rodents, bats (Calisher et al. 1983, Causey et al. 1961, Jonkers et al. 1968b) ArboCat*	Panama, Brazil, Trinidad	*Mammalphilic mosquito, Culex, Aedes, Mansonia, Limatus, Psorophora, Trichoprosopon, Lutzomyia*
		Ananindeua virus (ANUV)	Opossum, wild birds, rodents (Calisher et al. 1983), ArboCat*	Brazil	*Culex species*
		Moju virus (MOJUV)	Rodents (Walder, Suarez & Calisher 1984)	Brazil, Venezuela	*Culex sp., Mansonia, Sabethini sp., Psorophora*
		Mahogany Hammock virus (MHV)	Rodents (Coleman, Ryder & Work 1969)**	Florida	*Culex sp.*
	Timboteua virus	Timboteua virus (TBTV)	Rodents, ArboCat*	Brazil	Not isolated from arthropods

Koongol (Doherty et al. 1970, Stevenson, Holmes 1972, Mahy 2001)	*Koongol virus*	Koongol virus (KOOV)	Cattle, domestic fowl, wallabies, wild birds, and bandicoots (HI titres) (Doherty et al. 1963)	Australia, New Guinea	*Culex sp., Anopheles sp., Ficalbia ssp.*
		Wongal virus (WONV)	Cattle, domestic fowl, wallabies, wild birds, and bandicoots (HI titres) (Doherty et al. 1963)	Australia	*Culex annulirostris, Coquillettidia crassipes*
Minatitlan (Calisher et al. 1983)	*Minatitlan virus*	Minatitlan virus (MNTV)	- (Calisher et al. 1983)	Mexico, Guatemala	Not isolated from arthropods
		Palestina virus	- (Calisher et al. 1983)	Ecuador	*Culex paracrybda*
Nyando (Groseth et al. 2014)	*Nyando virus*	Nyando virus (NDV)	Humans (Groseth et al. 2014, Williams, Woodall & Corbet 1965)	Kenya, Uganda	*Anopheles funestus*
	Manéra virus **	Eret-147 virus (ERETV)	(Groseth et al. 2014)	Ethiopia, Cameron	*Aedimorphus dalzieli, Eretmapodites spp.*
	Kaeng Khoi virus	Kaeng Khoi virus (KKV)	Bats, humans (Groseth et al. 2014, Williams et al. 1976)	Thailand, Cambodia	bedbugs
	*Mojuı dos Campos virus***	BeAn 2761 isolate (MDCV)	bats (Groseth et al. 2014) ArboCat*	Brazil	Not isolated from arthropods
Olifantsvlei (Murphy, Harrison & Whitfield 1973)	*Olifantsvlei virus*	Olifantsvlei virus (OLIV)	- (Murphy, Harrison & Whitfield 1973, Ota et al. 1976)	South Africa, Sudan, Ethiopia	*Culex pipien, Cx. Poicilipes, Mansonia uniformis*
		Bobia virus (BIAV)	- ArboCat*	Central Africa	*Culex Tigripens*
		Dabakala virus (DABV)	- ArboCat*	Ivory coast	*Culex guiarti/ingrami*
		Oubi virus (OUBIV)	- ArboCat*	Ivory coast	*Culex (Eumelanomyia) rima group*
	Botambi virus	Botambi virus (BOTV)	- ArboCat*	Central Africa	*Culex guiarti*
Patois (Ushijima et al. 1980)	*Patois virus*	Patois virus (PATV)	Cotton rats (Srihongse, Galindo & Greyson 1966)	Panama	Not isolated from arthropods
		Abras virus (ABRV)	- (Calisher et al. 1983)	Ecuador	*Culex paracrybda, Cx. adamesi*
		Babahoya virus (PAHV)	- (Calisher et al. 1983)	Ecuador	*Culex ocossa*
		Shark River virus (SRV)	Cotton rats (Fields et al. 1969)	Florida, Mexico, Guatemala	*Culex sp., Culex nigripalpus, Anopheles crucians*

	Estero real virus	Estero real virus (ERV)	- (Malkova et al. 1985)	Cuba	*Ornithodoros tadaridae ticks*
	Zegla virus	Zegla virus (ZEGV)	Cotton rats, humans (Srihongse, Galindo & Greyson 1966, Scherer et al. 1972)	Panama, Mexico, Honduras, Guatemala	Not isolated from arthropods
Simbu (Kinney, Calisher 1981)	*Akabane virus*	Akabane virus (AKAV)	Cattle, sheep, goats, horses (Parsonson, Della-Porta & Snowdon 1977, Yang et al. 2008, Bryant et al. 2005, Parsonson, Della-Porta & Snowdon 1981)	Japan, Australia, Kenya, Vietnam, Korea	*Culicoides brevitarsis, Aedimorphus vexans, Culex tritaeniorhynchus*
		Sabo virus (SABOV)	Cattle, goats, sheep, swine (Causey et al. 1972)	Nigeria	*Culicoides*
		Tinaroo virus (TINV)	Ruminants (St George et al. 1979, Cybinski 1984)	Australia	*Culicoides brevitarsis*
		Yaba-7 virus (Y7V)	- (Mahy 2001)	Nigeria	*Mansonia Africana* mosquitoes
	Manzanilla virus	Ingwavuma virus (INGV)	Wild birds, pigs, buffalo, dogs, humans (Top et al. 1974, McIntosh, McGillivray & Dickinson 1965)	South Africa, Nigeria, Central African, India, Thailand, Cyprus	*Culex sp.*
		Manzanilla virus (MANV)	Monkeys (Anderson et al. 1960, Feng et al. 2015)	Trinidad, China	*Aedes sp., Culex sp.*
		Buttonwillow virus (BUTV)	Hares, rodents (Reeves et al. 1970)	USA	*Culicoides variipennis*
		Inini virus (INIV)	Pteroglossus aracari bird ArboCat*	French Guiana	Not isolated from arthropods
		Mermet virus (MERV)	Wild birds (Calisher et al. 1969, Calisher et al. 1981)	USA	*Culex restuans, Culex pipiens*
	Oropouche virus	Oropouche virus (OROV)	Humans, sloths, rodents monkeys, domestic and wild birds, (Pinheiro et al. 1981, Anderson et al. 1961)	Brazil, Peru, Panama, and Trinidad and Tobago	*Culicoides paraensis, Och. serratus, Culex quinquefasciatus, Mansonia sp*
		Facey's Paddock virus (FPV)	- (Doherty et al. 1979, Standfast et al. 1984)	Australia	*Culicoides, Culex annulirostris*
		Utinga virus (UTIV)	Sloths, humans (Seymour, Peralta & Montgomery 1983)	Brazil	Not isolated from arthropods

		Utive virus (UVV)	Sloths (Seymour, Peralta & Montgomery 1983)	Panama	Not isolated from arthropods
		Pintupo virus (PINV)	Sloths (Seymour, Peralta & Montgomery 1983)	Panama	*Culicoides diabolicus*
	Sathuperi virus	Sathuperi virus (SATHV)	Cattle (Causey et al. 1972, Yanase et al. 2004)	India, Nigeria, Japan	*Culicoides, Culex vishnui*
		Douglas virus (DOUV)	Cattle, ruminants, horses (St George et al. 1979)	Australia	*Culicoides brevitarsis*
	Shamonda virus	Peaton virus (PEAV)	Cattle, ruminants, horses, pigs, deer (St George et al. 1980, Matsumori et al. 2002)	Australia, Japan	*Culicoides brevitarsis*
		Shamonda virus (SHAV)	Cattle (Kinney, Calisher 1981, Causey et al. 1972, Yanase et al. 2005)	Nigeria, Japan	*Culicoides imicola pallidipennis*
		Sango virus (SANV)	Cattle, goat (Kinney, Calisher 1981, Causey et al. 1972)	Nigeria, Kenya	*Culicoides sp., Mansonia uniformis*
		Schmallenberg virus (SCHV)	Cattle, sheep, goats, dogs, deer (Hoffmann et al. 2012, Linden et al. 2012, Rasmussen et al. 2012, Sailleau et al. 2013)	Europe	*Culicoides sp*
	Simbu virus	Simbu virus (SIMV)	Humans (Kokernot et al. 1965, Smithburn et al. 1959)	South Africa, Senegal, Cameroun, Central African	*Aedes sp., Coquillettidia sp.,*
	Shuni virus	Shuni virus (SHUV)	Humans, cattle, sheep, horses (van Eeden et al. 2012, Causey et al. 1972)	Nigeria, South Africa	*Culicoides sp., Culex theileri*
		Aino virus (AINOV)	Cattle, horses (Yang et al. 2008, Noda et al. 1998, Doherty et al. 1972)	Japan, Australia, Korea	*Culicoides brevitarsis, Culex sp.*
		Kaikalur virus (KAIV)	- (Rodrigues et al. 1977)	India	*Culex tritaeniorhynchus*

	Thimiri virus	Thimiri virus (THIV)	Wild birds (Kinney, Calisher 1981, Carey et al. 1971)	India, Australia, Egypt	*Culicoides histrio*
	Oya virus††	Oya virus (OYV)	Pigs (Kono et al. 2002, Bryant et al. 2005, Zhang et al. 2013)	Malaysia, Vietnam, China	*Culex Quinquefasciatus, Culex sp., Anopheles spp., Mansonia sp.*
Tete (Calisher et al. 1990)	*Tete virus*	Tete virus (TETEV)	Wild birds (Anonymous1970d)	South Africa, Nigeria	Not isolated from arthropods
		Bahig virus (BAHV)	Wild birds (Hubalek, Rudolf 2012, Anonymous1970a)	Italy, Cyprus, Egypt	*Hyalomma marginatum* ticks
		Matruh virus (MTRV)	Wild birds (Hubalek, Rudolf 2012)	Italy, Cyprus, Egypt	*Hyalomma marginatum* ticks
		Tsuruse virus (TSUV)	Wild bird (Schaffer, Scherer 1972)	Japan	Not isolated from arthropods
		Weldona virus (WELV)	Wild birds, waterfowl (Calisher et al. 1990)	USA	*ceratopogonidae* midges
	Batama virus	Batama virus (BMAV)	Wild birds, ArboCat*	Central Africa	Not isolated from arthropods
Turlock (Klimas et al. 1981, Calisher et al. 1984)	*Turlock virus*	Turlock virus (TURV)	Wild birds, hares, horses (Lennette et al. 1957, Hayes, LaMotte & Holden 1967)	USA, Brazil, Canada, Trinidad	*Culex species*
		Umbre virus (UMBV)	Birds, humans, ArboCat*	India, Malaysia	*Culex species*
	M'Poko virus	M'Poko virus (MPOV)	- ArboCat*	Cameroon	*Ochlerotatus ingrami*
		Yaba-1 virus (Y1V)	- (Calisher et al. 1984)	Africa	
		Lednice virus (LEDV)	Waterfowl (Hubalek 2008, Malkova 1972)	Europe	*Culex modestus*
Wyeomyia ** (Chowdhary et al. 2012)	*Wyeomyia virus*	Wyeomyia virus (WYOV)	Humans (Sirhongse, Johnson 1965)	Colombia, Panama, Guiana	*Wyeomyia melanocephala* mosquitoes
		Anhembi virus (AMBV)	Humans (De Souza Lopes et al. 1975)	Brazil	*Phoniomyia pilicauda*
		BeAr 328208 =Cachoeira Porteira virus	- (Chowdhary et al. 2012)	Brazil	*Sabethes glaucadaemon*
		Macaua virus (MCAV)	Humans, ArboCat*	Brazil	*Sabethes soperi*
		Sororoca virus (SORV)	- ArboCat*	Brazil	*Sabethini spp.*
		Taiassui virus (TAIAV)	- (Chowdhary et al. 2012)	Brazil	*Sabethini spp.*

		Iaco virus (IACOV)	- ArboCat*and (Chowdhary et al. 2012)	Brazil	*Wyeomyia spp.*
		Tucunduba virus (TUCV)	Humans (Chowdhary et al. 2012)	Brazil	*Wyeomyia spp.*
Mapputta** (Gauci et al. 2015)		Maputta virus (MAPV)	Kangaroos, wallabies (Doherty et al. 1963, Doherty et al. 1970)	Australia	*Anopheles meraukensis*
		Maprik virus (MPKV)	Ruminants, pigs (Gauci et al. 2015)	New Guinea	*Aedes funereus*
		Trubanaman virus (TRUV)	Cattle, horses, kangaroos, wallabies (Anonymous1970e)	Australia	*Anopheles spp.*
		Gan Gan virus (GGV)	- (Doherty et al. 1979, Gard, Marshall & Woodroofe 1973)	Australia	*Aedes spp.*
		Buffalo Creek virus (BUCV; isolate DPP0186)	Human, cattle, pigs (Gauci et al. 2015)	Australia	*Anopheles meraukensis*
		Salt Ash virus (SASHV)	(Gauci et al. 2015, Coffey et al. 2014)	Australia	*Ochlerotatus vigilax*
		Murrumbidgee virus (MURBV)	(Gauci et al. 2015, Coffey et al. 2014)	Australia	*Anopheles annulipes*
Not classified to any serogroup					
	*Leanyer virus***	(LEAV) NT 16701 isolate	(Savji et al. 2011, Doherty et al. 1977)	Australia	*Anopheles meraukensis*
	*Termeil virus ** *	(TERV) BP8090	(Russell et al. 1991)	Australia	*Aedes vigilax, Cq. Linealis*
	*Sedlec virus isolate***	(SEDV) AV172	Reed warbler (Hubalek et al. 1990, Bakonyi et al. 2013)	Czechoslovakia	Not isolated from arthropods

† Mboke virus is a possible Ilesha virus isolate (Pachler, Ruzek & Nowotny 2013)
†† Oya virus possibly belongs to the Manzanilla virus complex (Feng et al. 2015)
* https://wwwn.cdc.gov/Arbocat/Default.aspx
**tentative serogroups or species after more sequencing data are available