Togaviridae

9

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Abstract

Alphavirus constitutes one of the two genera included in the family Togaviridae. This genus contains 31 viral species (with different variants and strains), grouped into seven antigenic complexes. Depending on the geographical location where they were isolated, this genus is divided into alphavirus in the New World (including Eastern equine encephalitis virus, Venezuelan equine encephalitis, and Western equine encephalitis, which cause encephalitis in humans and other mammals) and the Old World (chikungunya virus, o'nyong-nyong virus, Ross River virus, Semliki Forest virus, and Sindbis virus causing syndrome characterized by fever, rash, and arthralgias, which rarely cause mortality). However, Sindbis virus (the prototype alphavirus) causes encephalomyelitis in mice, and Ross River virus and chikungunya virus (CHIKV) are also neuroinvasive and cause neurological disease in humans. Alphaviruses are responsible for several medically important emerging diseases and are also significant veterinary pathogens. Due to the aerosol infectivity of some alphaviruses and their ability to cause severe, sometimes fatal neurological diseases, they are also of biodefense importance. Likewise, they are of interest for their potential use in gene therapy. This chapter will describe general aspects of alphavirus, with emphasis on their pathology, ecology, epidemiology, clinical, diagnosis, treatment, prevention, and control measures.

Keywords

Alphavirus • Equine encephalitis • Chikungunya

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9.1 Introduction

The family Togaviridae includes two genera: *Rubivirus* (rubella virus, which only infects humans and is maintained by human-to-human transmission) and *Alphavirus* (arboviruses) (ICTV 2014). Most of the last are mosquito-associated arboviruses but some are tick borne.

Many alphaviruses are of human and veterinary concern because they cause diseases of public health and economic importance (Hubálek et al. 2014). Clinical symptoms caused by alphavirus infection vary according to the viral species (Griffin 2007).

Alphavirus genera account for a total of 31 viral species (ICTV 2014). Their members have been detected in all continents, except for Antarctica, mainly due to the absence of an arthropod vector. Many alphaviruses were firstly isolated in tropical regions from Africa and South America and in some Asian countries (Calisher and Karabatsos 1988). Several factors, such as changes in viral genetics, host and/or vector population, and climate changes, facilitated the expansion and transmission of alphavirus in the past several decades.

Based on their original geographic distribution, alphaviruses are clustered into two main groups: New World and Old World. The former includes mainly neurotropic alphaviruses pathogenic for humans and equines (i.e., Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis complex viruses). The exception is the Mayaro virus, which is associated with febrile illness and arthralgia (a dengue- and chikungunya-like disease).

The Old World alphaviruses are mainly associated with febrile illness, eruption, and arthralgia in humans. They rarely cause death (i.e., chikungunya virus, CHIKV; o'nyong-nyong virus, ONNV; Ross River virus, RRV; Getah virus, GETV; Middelburg virus, MIDV; Semliki Forest virus, SFV; Sindbis virus, SINV). GETV, MIDV, SFV, and SINV can cause neurological affections in equines (Table 9.1) (Griffin 2007).

9.2 Viral Agent

The prototype virus in the genera is SINV. All members have a similar virion structure, replication strategy, and molecular characteristics (Griffin 2007).

Table 9.1 Biological features and geographic distribution of virus belonging to *Alphavirus* genus

Antigenic complex	Viral specie	Antigenic subtype	Antigenic variety	Associated clinical syndrome	Distribution
Barmah Forest	Barmah Forest virus (BFV)			EFA	Australia
Eastern equine encephalitis (EEE)	EEEV	I, II, IV		EFE	North America and Caribbean
	Madariaga virus	III			South and Central America
Middelburg	Middelburg virus (MIDV)			ENR	Africa
Ndumu	Ndumu virus (NDUV)			ENR	Africa
Semliki Forest	Semliki Forest virus (SFV)			EF	Africa
	Chikungunya virus (CHIKV)			EFA	Africa, Asia, Europe
	O'nyong-nyong virus (ONNV)			EFA	Africa
	Getah virus (GETV)			ENR	Asia
	Bebaru virus (BEBV)			ENR	Malaysia
	Ross River virus (RRV)	Sagiyama		EFA	Australia, Oceania
	Mayaro virus (MAYV)			EFA	Central and South America, Trinidad
	UNA virus (UNAV)			ENR	South America

(continued)

Table 9.1 (continued)

Antigenic complex	Viral specie	Antigenic subtype	Antigenic variety	Associated clinical syndrome	Distribution
Venezuelan equine encephalitis (VEE)	VEEV	I	AB	EFE	American continent
			C		Northern South America
			D		South and Central America
			E		Mexico and Central America
	Mosso das Pedras virus (MEDV)		F	EFE	South America (Brazil)
	Everglades virus (EVEV)	II		EFE	Florida (USA)
	Mucambo virus (MUCV)	III	A, C, and D	EFE	South America
	Tonate virus (TONV)		B		Brazil, USA
	Pixuna virus (PIXV)	IV		ENR	Brazil, Argentina
	Cabassou virus (CABV)	V		ENR	French Guyana
	Rio Negro virus (RNV)	VI		ENR	Argentina
Western equine Encephalitis (WEE)	Sindbis virus (SINV)	Sindbis		EFA	Africa, Europe, Asia, Australia
		Babanki		EFA	Africa
		Ockelbo		EFA	Europe
		Kyzylagach		ENR	Azerbaijan, China
	Whataroa virus (WHAV)			ENR	New Zealand
	AURA virus (AURAV)			ENR	South America
	WEEV	Several		EFE	West coast of North America, South America
	Highlands J virus (HJV)			ENR	East coast of North America
	Fort Morgan virus (FMV)	Buggy Creek		ENR	West coast of North America
Trocara	Trocara virus (TROV)			ENR	South America
Eilat virus		I–V		ENR	Israel
	Salmon pancreas disease virus (SPDV)			Pancreatic Disease (Salmon)	Atlantic Ocean
	Sleeping disease virus			Sleeping disease (trout)	Europe
	Southern elephant seal virus (SESV)			ENR	Australia

The alphaviruses are enveloped, single positive-stranded RNA viruses with icosahedral symmetry. Virions are 60–70 nm in diameter and are sensitive to ether and chloroform (Fig. 9.1a). Viral genome is organized in two modules: two thirds of the methylated 5′ end encodes for non-structural proteins (nsPs). The polyadenylated 3′ end third encodes the structural proteins (E1, E2, E3, and 6K) (Fig. 9.1b). Nonstructural proteins have a key role during transcription and replication of viral RNA. Viral genome (10–12 kb length) is included inside the viral capsid made by C protein (capsid protein). Both elements compose the nucleocapsid surrounded by a lipoprotein envelope. The viral envelope is a host cell-derived structure on which glycoproteins E1

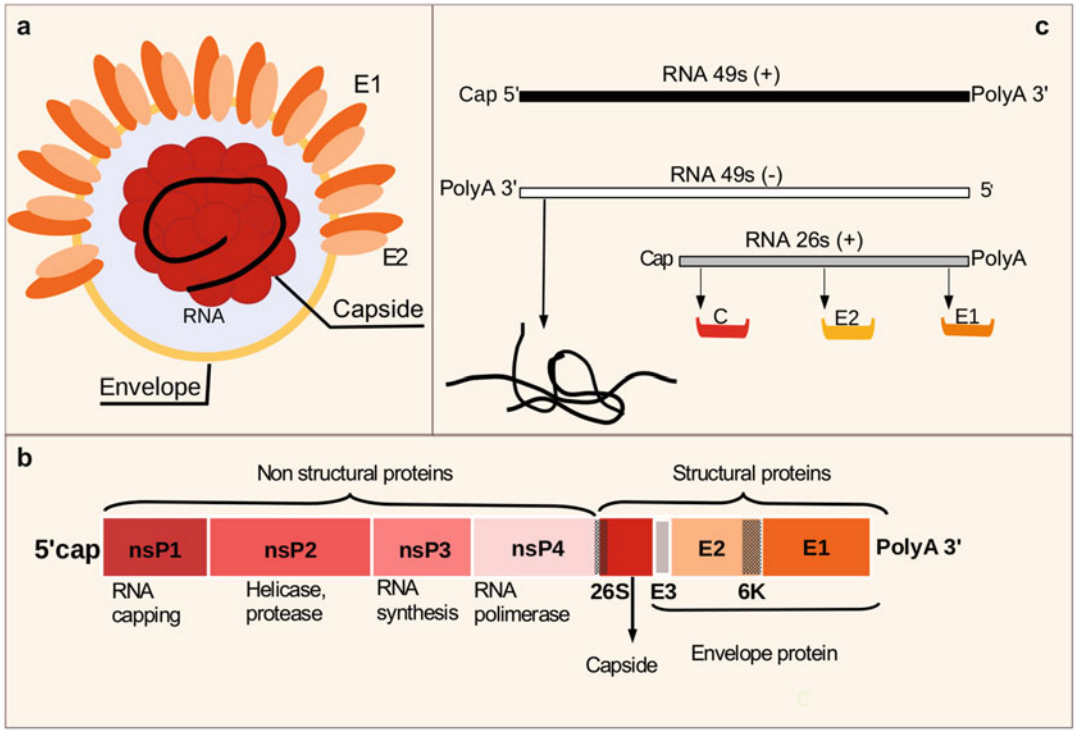

Fig. 9.1 A schematic representation of an *Alphavirus* virion (**a**) and its RNA genome (**b**) alphavirus replication cycle (**c**)

and E2 are embedded. The E2 glycoprotein has main epitopes for neutralizing antibodies (Griffin 2007, Jose et al. 2009).

Alphavirus virions have the ability to agglutinate avian red cells. This trait is based on E1 and E2 proteins and has been used for viral quantification. It also allows the use of the hemagglutination inhibition assay (HIA) for the serological screening in alphavirus infections. Neutralizing antibodies react to epitopes localized mainly in E2 protein and are viral type specific. The HIA has been an extremely useful technique for analyzing phylogenetic relationships and clustering alphavirus in serogroups. Up to date, eight serocomplexes have been detected in the *Alphavirus* genera, including the latest Trocara virus complex. The BF, EEE, MID, NDU, and TROC serogroups have only one viral member, whereas other serogroups include several viral species (Table 9.1). Moreover, subtypes can be identified for particular viral species (Calisher and Karabatsos 1988, Powers et al. 2001).

At the molecular level, the C and E1 amino acid sequences are more conservative than the E2 protein, and, in general, antigenic clusters overlap with the molecular classification.

Viral species are defined by a combination of genetic, ecological, and antigenic information. In general, they have a different transmission cycle and 23 % and 10 % of nucleotide and amino acid divergence, respectively (Griffin 2007).

9.3 Replication Cycle

Viral infection in a vertebrate host cell starts with the inhibition of the macromolecular synthesis of the cell. The cell biosynthetic mechanism is driven by virus proteins and converted into a viral particle factory.

The viral RNA acts as a messenger RNA in the production of viral nsPs. It is also transcripted by a viral polymerase into a negative-sense complementary strain RNA, which is used as a

template during the synthesis of subgenomic RNA 26S and 49S (Fig. 9.1c). The smaller subgenomic RNA encodes for structural proteins. Nucleocapsids are produced in the cytoplasm and released by rotation through the plasmatic membrane of the cell host.

Alphaviruses can replicate in a wide range of vertebrate species and types of cells. Suckling albino mice are highly susceptible to most alphaviruses. These viruses are also well isolated and replicate in a wide variety of cellular cultures, such as primary chick embryo fibroblast, Vero (green monkey kidney), BHK (suckling hamster kidney), and C6/C36 (mosquitoes) (Griffin 2007).

9.4 Neurotropic Alphaviruses

9.4.1 Eastern Equine Encephalitis Virus (EEEV)

9.4.1.1 Viral Pathogenesis in Humans and Equines

Most EEEV infections in *humans* are inapparent. In those patients who get sick, signs and symptoms start between 3 and 10 days postinfection and the disease lasts from 1 to 2 weeks. Symptoms range from febrile illness, with cephalea and myalgia, followed by recovery to encephalitis, coma, and death. Death generally occurs 2–10 days after the onset of encephalitis (Go et al. 2014).

Cerebrospinal fluid (CSF) can appear clear, with white blood cells (2–2000 μl), with predominance of polymorphonuclear leukocytes, and the disease starts with replacement of mononuclear cells. CSF protein level is high, whereas glucose is normal or low. Blood can be observed in CSF. Electroencephalogram analyses can provide additional indirect evidence of EEEV infection. Computed tomography examination can be normal or show only edema, whereas magnetic resonance images are frequently abnormal and show focal lesions commonly in the basal ganglia, thalamus, and brainstem (Steele and Twenhafel 2010).

EEEV represents the most virulent and pathogenic alphavirus in the USA, being a serious concern for the public health. Mortality rate ranged from 30 to 40 %. People under 10 years old are the most susceptible to develop encephalitis and death. The highest mortality rates are observed in elderly population as well. Sequelae like paralyses and mental retardation are observed in 35–80 % of survivors, particularly in children.

EEEV causes encephalitis in *equines*. Disease starts with fever, anorexia, and colic followed by virus invasion of CNS, causing encephalitis and myelitis associated with abnormal behavior (stepping, disequilibrium, tendency to walk in circles), somnolence, paralysis, and seizures before death. Mortality rate ranges from 80 to 90 %. In the USA, the disease has been detected in pheasants, turkeys, dogs, and pigs. Birds generally do not get sick but develop high viremias. Those birds that get sick and die develop systemic illness with viscerotropic compromise with no encephalitis except for pheasants, which develop encephalitis. Recently, wild deers (*Odocoileus virginianus*) with neurological disorders have been detected to be infected with EEEV. This finding has placed this virus as a concern for wildlife conservation (Zacks and Paessler 2010).

9.4.1.2 Epidemiology

EEEV is widely distributed in the Americas. It has been detected all along the eastern coast, from Canada and the USA, through the Gulf of Mexico and Central American and Caribbean islands, to South America (Go et al. 2014).

In the USA, this virus is a main cause of neurological disease in domestic animals and humans. Severe disease is observed in humans, pigs, dogs, equines, and pheasants. Diseases by EEEV in equines were first recognized in 1931 in the USA. However, some reports of encephalomyelitis outbreaks from 1845 to 1912 are available (Scott and Weaver 1989). The first EEEV strain was isolated from the brain of an ill horse in New Jersey and Virginia during a wide extended epizooty in 1933. Moreover, the first fatal human cases were reported at the same places where equine encephalitis cases were documented in 1938. Although human cases by EEEV are not common in the USA, the average annual cases amount to 5 (ranging between 0 and

15). The states of Florida, Georgia, Massachusetts, and New Jersey have a high number of cases. Recently, human outbreaks of encephalitis were reported in northern states along the east coast of the USA (New Hampshire and Maine) and Canada. Likewise, simultaneous EEEV and VEEV outbreaks were reported in 2010 in Panama (Lubelczyk et al. 2013, Molaei et al. 2013, Vander Kelen et al. 2012, Yu et al. 2015).

In South America, EEEV was first isolated during an equine encephalitis outbreak in Argentina in 1936. Numerous epizootics by EEEV, some of them with thousands of cases, have been reported in Argentina, Brazil, Colombia, and Venezuela. The last EEEV epizootic reported in Argentina was in 1988. In Brazil, new equine encephalitis cases from southeastern areas were recorded during 2003, 2006, and 2009. During the equine epizootics in Argentina and Brazil, the high seroprevalence was detected in human population without human clinical cases. Up to date, only human cases were reported in Brazil (1956) and Trinidad (1970) (Sabattini et al. 1998, Scott and Weaver 1989, de Novaes Oliveira et al. 2014).

The comparison of EEEV strains from North and South America shows molecular, epidemiological, and ecological differences. Based on molecular evidences, four lineages can be distinguished: lineage I detected in North America and three lineages detected in South America (lineage II, Brazil, Guatemala, and Peru; lineage III, Argentina, Brazil, Colombia, Equador, Guayana, Panama, Peru, Trinidad, and Venezuela; lineage IV, Brazil). These clusters show that South American strains are more diversified and heterogeneous and that their antigenicity and geographic distribution are correlated. North American strains are associated with high virulence in humans and equines, whereas South American strains are pathogenic for equines, being attenuated for humans (Bhavez et al. 1999, Young et al. 2008, Weaver et al. 2012).

In North America, EEEV is endemically maintained by birds and ornithophilic mosquitoes (*Culiseta melanura*) in freshwater swamps. Under favorable ecological and environmental conditions, the virus can be transmitted to humans and domestic animals. Several mosquito species (e.g., *Aedes*[1] *sollicitans*, *Culex salinarius*, *Ae. canadensis*) with a wide host preference range have been incriminated as bridge vectors (Fig. 9.2). In the USA, human and equine cases are seasonal (late summer-early fall); however, in southeastern areas cases are reported year-round. The overwinter mechanism in the temperate area is unknown. Annual reintroduction through migrating birds and/or infecting mosquitoes from subtropical areas has been postulated. On the other hand, no transovarian transmission has been documented (Weaver et al. 2012, Go et al. 2014).

In the Caribbean and Central and South America, viral transmission occurs endemically, and several mosquito species are involved as enzootic vectors. Epidemiological data is scarce because of the lack of human cases by EEEV infection. Multiple isolations were made from *Culex (Melanoconion)*[2] spp. mosquitoes collected in Central and South America. Serological studies indicate that small mammals and birds would serve as maintenance host. Moreover, bats and marsupials infected by EEEV were detected (Go et al. 2014).

In Venezuela, EEEV enzootic strains were isolated from sentinel hamsters in 1975 and from equines in 1976 during an epizootic period. Circulating strains belong to lineage III.

In Brazil, equine epizootic events were reported in north, northeastern, and southeastern areas. Viral strains have been isolated from monkeys, chickens, sentinel hamsters, birds, mosquitoes (*Culex* spp., *Ae. taeniorhynchus*), and ill equines. The three South American lineages circulate in Brazil and are maintained by birds and rodents (hosts) and the mosquitoes *Culex pedroi* (enzootic vector) and *Ae. taeniorhynchus* (epizootic vector). In 2003, two equine encephalitis

[1]Although several taxonomic modifications have been proposed on Culicidae genera, mostly splitting *Aedes* by Reinert et al. (2009) (and accepted by CBM), this taxonomy has been used in the chapters according to authors' preference. To facilitate utilization by health personnel, all new aedine genera can be considered *Aedes* (CBM).

[2]This is a group of mosquitoes (160 spp.) particularly difficult to identify (CBM).

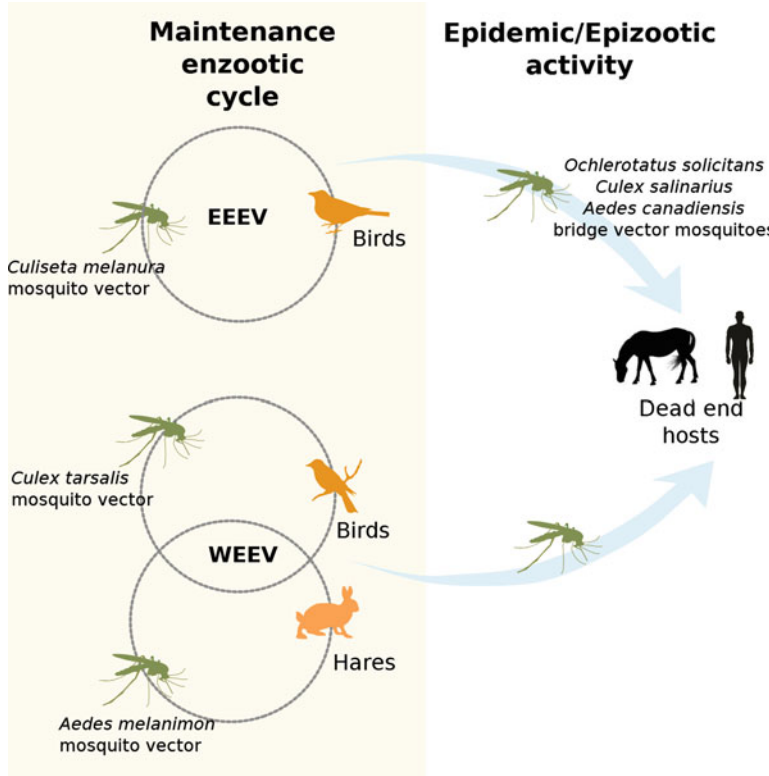

Fig. 9.2 Maintenance and transmission cycles for Eastern and Western equine encephalitis viruses

cases were reported in the southeastern region. The two last encephalitis cases in equines have been detected in 2006. Only one human case was reported 50 years ago.

In Argentina, EEEV was first isolated in a sick horse in Buenos Aires province in 1930; however, the virus was identified in 1953. EEEV has produced important periodic epizooties. Although mixed epizooties (EEEV/WEEV) have occurred, most of them have been produced by one viral agent. This recognized situation in Argentina is exceptional and has only been observed in Guayana. So far, EEEV has never been isolated from mosquitoes and its possible vector in the maintenance cycle is unknown. Serological studies incriminate wild and domestic birds in its viral cycle. Paradoxically, no infected bird was detected during equine epizootics in Santiago del Estero (1981) and Chaco (1988) provinces, suggesting that maintenance cycle occurred elsewhere or that migratory birds could have been involved (Weaver et al. 2012).

9.4.2 Western Equine Encephalitis Virus (WEEV)

The WEEV along with six viral species makes up the Western equine encephalitis antigenic complex (Table 9.1). WEEV, Highlands J virus (HJV), Fort Morgan virus (FMV), and AURA virus (AURAV) circulate in the Americas, each of them showing a particular ecological niche. HJV, WEEV, and FMV belong to a lineage that originated from recombination of a Sindbis-like virus and EEEV (Zacks and Paessler 2010, Go et al. 2014).

9.4.2.1 Pathogenicity in Humans and Animals

Neurological diseases caused by WEEV have signs and symptoms similar to those of EEEV, but less severe. The spectrum ranges from asymptomatic infection to lethal forms, with intermediate stages characterized by fever, headache, or aseptic meningitis. In the USA, an asymptomatic/symptomatic infection ratio is strongly age

dependent, being more severe in young children. The initial symptoms are fever, chills, headache, nausea, and vomiting. Respiratory symptoms are occasional and may last from 3 to 5 days; then, the neurological signs and symptoms appear (tremors, lethargy, irritability, stiff neck, photophobia, dizziness, and altered mental status). CSF shows pleocytosis with 100–1500 cells/μl predominantly neutrophils at first and subsequently with mononuclear cells. Most cases have complete recovery. In adult individuals, remission of signs and symptoms occurs between 5 and 10 days, recovery is generally rapid, and sequelae are rare. In children under 1 year of age, 60 % of survivors have brain damage, and in some of them, the disease is progressive with persistent infection.

In *equines* (horse, mules, and ponies), the disease is characterized by fever and encephalitis, which is often manifested as depression, anorexia, and paralysis of the lips and legs. The incubation period is 3–12 days. This is the form of the disease known as "lethargic"; the sick animal may or may not progress to the so-called "furious" form, which is where the vulgar designation "crazy horse" is derived, and it is how this disease is known in the field.

Signs of CNS involvement include falling head or ears, chewing movements, excessive salivation, incoordination, locomotion in circles, inability to stand, flabby lips, apparent blindness, ataxia, involuntary movements, irritability, hyperexcitability, and seizures. In the terminal state, side-lying prostrate animals show pedaling movements, nystagmus, paralysis, difficult breathing, and coma (Griffin 2007, Steele and Twenhafel 2010).

9.4.2.2 WEEV Distribution and Ecoepidemiology

WEEV is distributed from Canada to Argentina and is the only member of the complex pathogenic for humans. Epizootic and enzootic viral subtypes have been recognized for this virus. Viral strains isolated from ill horses and humans are considered pathogenic strains (epizootic strains), whereas those isolated from mosquitoes during interepizootic periods are attenuated for humans and equines (enzootic strains). Four viral lineages have been identified, two lineages endemic to South America and the other two widely distributed in the Caribbean and North and South America.

WEEV was isolated for the first time during equine epizootics in California (USA) in the summer of 1930. Previous evidences suggest that thousands of equines would have died by this virus in 1912. After that, in 1938 WEEV was isolated from the brain of a fatal human case confirming its virulence for humans. In Argentina, similar facts were reported: in 1908 an equine encephalitis outbreak took place and in 1933 public health personnel isolated the virus from an ill horse.

In the west coast of the USA, the transmission cycle includes *Cx. tarsalis* mosquitoes as enzootic and epizootic/epidemic vector and Passeriformes birds (mainly house sparrows, *Passer domesticus*) as primary amplifying hosts. Secondary amplifying hosts which *Cx. tarsalis* frequently feed on include Passeriformes birds, chickens, and pheasants. An alternative *Cx. tarsalis* bird transmission cycle can occur between *Ae. melanimon* mosquitoes and black-tailed jackrabbit (*Lepus californicus*). The latter develops a nonfatal infection with long viremia. Humans, equines, and other mammals are dead-end hosts (Fig. 9.2). Although *Cx. tarsalis* is mainly considered an ornithophilic vector, its host feeding preference changes from summer to fall, with mammals becoming the most frequent host. This evidence suggests the role of *Cx. tarsalis* as a bridge host, driving viral activity from birds to humans during early fall.

Viral isolations and serological studies have detected natural infections in chickens and other domestic birds, rodents, rabbits, reptiles, and amphibians; these two last animals are suspected to act as overwinter hosts for WEEV in temperate regions. In some regions of South America, most mosquito species of which WEEV have been isolated are mammalophilic. Small mammals (rodents and rabbits) are frequently found infected by WEEV. The opposite is observed in other areas where birds are frequently seropositive.

In the temperate area of Argentina, important equine epizooties were recorded from the beginning of the twentieth century, simultaneously

with that caused by WEEV. Equine epizootics were recorded regularly every 5–10 years, with a mortality rate of 1–40 %. The last outbreak occurred in the summer of 1988–1989. During interepizootic periods, sporadic equine cases were reported, suggesting small focal outbreaks or iatrogenic cases due to inadequately inactivated vaccines. During the 1982–1983 epizootic, epizootic viral strains were isolated from *Ae. albifasciatus*, *Anopheles albitarsis*, *Mansonia* spp., and *Psorophora pallescens* mosquitoes. In the years preceding this epizooty, enzootic strains were isolated from *Cx. (Melanoconion) ocossa*. This prototype enzootic strain (AG80-646) is attenuated for equines, which shows it is different from WEEV. The natural transmission cycle is supposed to be integrated by *Cx. (Mel.) ocossa* mosquitoes and cricetid rodents, in which antibodies have been found (Fig. 9.3). For epizootic viral strains isolated in Argentina, the existence of two transmission cycles is assumed. The primary cycle might be called maintenance cycle, with unknown vectors and hosts, which would be analog to that observed in the USA between *Cx. tarsalis* mosquitoes and house sparrows. The other one, called amplifying cycle, has *Ae. albifasciatus* mosquitoes and mammals of Caviidae and Leporidae families as potential hosts. In this cycle, birds have had no apparent role in the maintenance of WEEV, since none or low seroprevalence was detected. Experimentally inoculated horses developed viremias high enough to infect vector mosquitoes, indicating their potential role as hosts.

Besides equine encephalitis epizootics reported in Argentina, outbreaks have been documented in Brazil and Paraguay (Go et al. 2014, Hubálek et al. 2014).

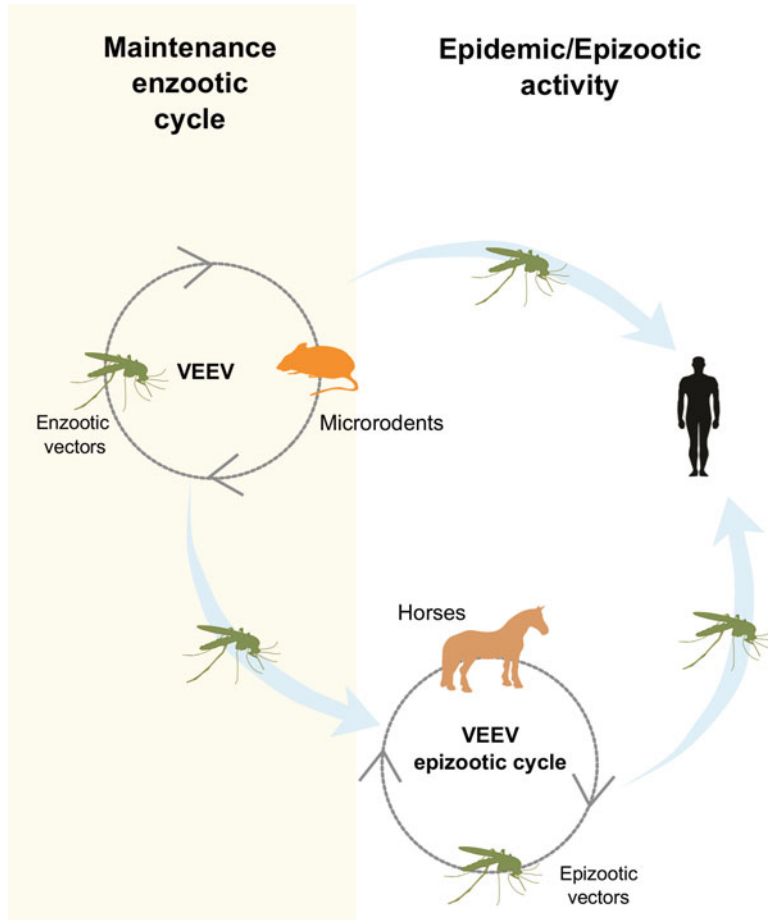

Fig. 9.3 Maintenance and transmission cycle of enzootic and epizootic subtypes of Western equine encephalitis virus

9.5 Other WEEV Virus Complex in the Americas

9.5.1 Highlands J Virus (HJV)

The Highlands J virus (HJV), identified in the eastern USA, was initially considered a variant of WEEV, but now is considered a separate entity. It was isolated from a corvid (*Cyanocitta cristata*) in Florida in 1960. This virus is enzootic in the eastern USA and is maintained by *Cs. melanura* as the primary vector and migratory birds as primary hosts. HJV may occasionally cause encephalitis in horses but is recognized as pathogenic for turkeys, pheasants, partridges, ducks, and other birds (Weaver et al. 1997, Griffin 2007, Steele and Twenhafel 2010).

9.5.2 Fort Morgan Virus (FMV)

The FMV and its closely related Buggy Creek virus (BCV) are not recognized as pathogenic for humans and pets. Both were isolated from swallows and sparrows in eastern Colorado and Oklahoma. It is interesting to note that BCV strains were isolated from bedbugs (*Oeciacus vicarius*) collected from bird nests during the summer in Colorado. These bugs could keep the virus during the winter and transmit it to the newborn birds in the early spring to restart the bird-mosquito cycle (Griffin 2007, Allison et al. 2015).

9.5.3 AURA Virus

The AURA virus was isolated from *Culex (Melanoconion)* sp. and *Ae. serratus*[3] later in northern Brazil in 1959. In Argentina, it was isolated from *Ae. serratus* captured in the province of Misiones in 1966. There is no evidence of a pathogenic role for humans. This virus is serologically related to Sindbis virus (found in the Old World) and WEEV (present in the Americas).

[3]This is a species complex, whose females are very difficult to differentiate (CBM).

AURAV shares substantial sequence identity and genome organization with Sindbis virus and important antigenic epitopes are preserved in both viruses. However, despite their close relationship, there is a significant divergence, sharing 73 % of the amino acid sequence of the nonstructural proteins and 62 % identity in structural proteins. By contrast, WEEV glycoproteins are more closely related to Sindbis virus than to AURA virus.

AURA virus has been demonstrated to be the least neurovirulent virus for mice among the enzootic viruses in WEEV complex. A characteristic demonstrated for the virus isolated in Argentina is the ability to replicate and produce experimental myocarditis in mice.

The geographic distribution of AURA virus obtained from epidemiological surveys in Argentina is greater in subtropical provinces (Chaco and Corrientes) than in temperate ones (Cordoba, Santa Fe, and Santiago del Estero) (Sabattini et al. 1998, Griffin 2007).

9.5.4 Venezuelan Equine Encephalitis Virus Complex

Venezuelan equine encephalitis virus (VEEV) is an important human and animal zoonotic pathogen, which causes periodic outbreaks of highly debilitating disease in the Americas. VEEV was first isolated in the Guajira region, Venezuela, from the brain of a sick horse during an outbreak of equine encephalitis in 1938. In the following years, VEE-related viruses were isolated and identified in many locations in South America, Central America, the Caribbean islands, and the southern regions of the USA. Isolates of these viruses related to VEEV were originally classified into subtypes I through IV, forming the VEEV complex by using a short incubation hemagglutination inhibition (HI) test. Subsequently, Cabassou virus (CABV) and Rio Negro virus (RNV) were isolated and were found to be within VEEV antigenic complex, becoming subtypes V and VI, respectively. Hence, VEEV complex consists of six serological subtypes, which are currently considered as viral species, differing in

their epidemiological characteristics and pathogenicity for humans and Equidae (mainly horses) (Table 9.1) (Steele and Twenhafel 2010, Weaver and Reisen 2010, Hubálek et al. 2014).

The analysis of the genetic relationships of different viruses included in VEEV complex has been consistent with the classification scheme in serocomplex. However, recent analyses of the nucleotide sequence of the 3′ terminal region that encodes the nsP4 and the complete 26S mRNA sequence of the genome region have suggested some rearrangements. Thus, the virus subtype II (EVE) would be more related to the IAB, IC, ID, and IE subtypes, whereas subtype IF would be more related to Rio Negro virus (subtype VI) isolated only in Argentina to date.

VEEV subtypes are divided into two epidemiological groups: epidemic/epizootic viruses and enzootic viruses (Table 9.2). Subtypes IAB and IC belong to the group of epidemic/epizootic viruses, which emerge periodically causing outbreaks that affect humans and equines, with high morbidity and mortality rates.

Since the isolation and characterization of VEEV in Venezuela in 1938 during an epidemic in horses, sporadic epidemics have been reported. Among them we can highlight the one that occurred between 1969 and 1972, affecting South and Central America, Mexico, and Texas, and the epizootic/epidemic in 1995 in Colombia and Venezuela after more than 20 years of silence, with about 50,000 equine cases and 75,000 human cases (Aguilar et al. 2011).

In Argentina, there are records of epizootic VEEV strains isolated from sick horses. However, the origin of these cases is not clear, and they may have been introduced by inactivated vaccines produced by private laboratories. Handling of these strains has caused laboratory infections, some of them serious.

Table 9.2 Members of the Venezuelan equine encephalitis virus complex

Serologic classification (subtype)	Virus/prototype strain	Origen	Source	Current distribution
IAB	VEEV – Trinidad donkey	Trinidad, 1943	Donkey	North, Central, and the north of South America
IC	VEEV – P676	Venezuela, 1963	Mosquito	North of South America
ID	VEEV – 3880	Panama, 1961	Human	South and Central America
IE	VEEV – Mena II, 68U201	Guatemala, 1968	Hamster	Central America and Mexico
IF	Mosso das Pedras virus – 78 V3531	Brazil, 1978	Mosquito	Brazil
II	Everglades virus (EVEV) – Fe3-7c	Florida, 1963	Mosquito	Florida (USA)
IIIA	Mucambo virus (MUCV) – BeAn8	Brazil, 1954	Monkey	North of South America and Trinidad
IIIB	Tonate virus – CaAn410d	French Guiana, 1973	Birds	French Guiana
IIIC	Bijou Bridge virus	Colorado, 1974	Mosquito	West of the USA (Colorado)
IIID	MUCV – 71D1252	Peru, 1971	Mosquito	Peru
	MUCV – V407660	Peru, 1998	Rodent, mosquito, and human	Peru
IV	Pixuna virus (PIXV) – BeAr35645	Brazil, 1961	Mosquito	Brazil
V	Cabassou virus (CABV) – CaAr508	French Guiana, 1968	Mosquito	French Guiana
VI	Rio Negro virus (RNV) – AG80-663	Argentina, 1980	Mosquito	Argentina

Transmission cycle for these subtypes involves equines, which act as highly efficient amplification hosts, developing high viremia titers, and mosquito vectors (Fig. 9.3). The equine virulence and the viremia induction are the most important labels of the epizootic phenotype (Weaver et al. 2004, Greene et al. 2005, Griffin 2007).

Although infection by epidemic/epizootic strains has been observed in humans, sheep, dogs, bats, rodents, and some birds, no major epidemics have been recorded in the absence of equine outbreak. Humans develop substantial viremia titers, but they probably do not act as amplifying hosts due to a lower exposure to mosquito bites. Because of this, transmission by humans should not be discarded. Infected people develop high titers of virus with both epizootic and enzootic strains (Weaver et al. 2004).

The epidemic/epizootic strains are opportunistic in the use of mosquito vectors during outbreaks. Field studies have indicated that more than one mosquito species can be involved in viral transmission during an outbreak. The vectors involved for this virus are mosquitoes of the genera *Aedes* and *Psorophora* including mosquitoes with peridomestic urban habits, such as *Ae. aegypti* and *Ae. albopictus*. Therefore, the requirements for the occurrence of human-mosquito-human transmission are met. *Aedes sollicitans* and *Ae. taeniorhynchus* also exhibit high infection rates, depending on the region of the Americas. *Aedes taeniorhynchus* is probably the most important epizootic vector in South America (Weaver et al. 2004, Weaver and Reisen 2010, Aguilar et al. 2011).

9.5.4.1 Pathogeny in Humans and Other Animals

Enzootic strains produce mild clinical disease, whereas the clinical forms of epizootic virus infection can be serious. The infection entry is generally through the skin, mosquito bites, but may occur through the airway (human cases of infection by aerosols have been recorded in laboratory accidents). The disease is almost undistinguishable clinically from other viral diseases such as influenza and dengue. Moreover, several Venezuelan equine encephalitis infections at early stage have been diagnosed as dengue. The disease usually begins abruptly 2–5 days after exposure (sometimes the incubation period is only 1 day), with chills, severe headache, fever, myalgia, retro-orbital pain, nausea, vomiting, and sore throat. Eighty percent of the infections are mild and last only 3–5 days. In many cases the febrile course is biphasic.

Neurological symptoms appear with headache and vomiting 4–10 days after the onset of clinical symptoms. Central nervous system disorders ranged from drowsiness to frank encephalitis with disorientation, convulsions, paralysis, coma, and death. This condition of the central nervous system is most common in children. Five percent of VEEV-infected children under 15 years old may develop neurological conditions, whereas in children of less than 5 years of age, this estimation may reach 35 % of infected individuals. In many cases there are sequelae, such as mental retardation, epilepsy, learning difficulties, hydrocephalus, personality changes, and paralysis. In young adults, the disease is relatively benign. Adults over 50 years of age are more likely to develop encephalitis.

Laboratory analyses often show leukopenia. The pathological damages observed in fatal cases are myocarditis, focal hepatic necrosis, inflammation, and generalized lymphoid depletion. In pregnant women infected during pregnancy, an increase in abortions and children born with congenital malformations (especially at the central nervous system level) was found.

When enzootic strains infect *horses*, they produce asymptomatic infections or cause a short fever, low level of viremia, and mild clinical symptoms. Enzootic virus infection can immunize horses against epizootic strains. The clinical symptoms produced by epizootic strains is characterized by fever, depression, and diarrhea, leading to death 6–8 days after infection. The animals have viremia titers well above the vector infection threshold. The virus can be recovered from ocular and nasal lavage and urine. Progressive leukopenia is observed until death. Fatalities in pancreatic necrosis and cell depletion in the bone marrow, lymph nodes, and spleen were observed. The brain of horses with encephalitis

has inflammation in cerebrovascular endothelial cells, edema, and extravasation of blood and leukocyte infiltration (Zacks and Paessler 2010, Taylor and Paessler 2013).

9.5.4.2 Treatment

Treatment of these diseases is only symptomatic, since no specific treatment is available.

9.5.4.3 Origin of Epidemics

Venezuelan equine encephalitis epidemics or epizootics have occurred in intervals of approximately 10–20 years in areas with livestock in many places of South America, when epizootic mosquito populations increase. For several years, the main enigma regarding VEEV epidemiology was to detect the source of epidemic/epizootic virus and the mechanisms of persistence between outbreaks. To explain this, several hypotheses have been postulated: (a) the emergence of epizootic subpopulations within enzootic VEEV populations, (b) the initiation of outbreaks by the administration of improperly inactivated vaccines, (c) the emergence of subtypes IAB and IC from cryptic transmission cycles, (d) the maintenance of epizootic strains in latent infections in horses or other animals, and (e) the periodic emergence of epizootic IAB, IC, and IE strains from the evolution (mutations) of enzootic VEEV progenitors. The last hypothesis has been supported by extensive phylogenetic analyses and is the most accepted so far. Remarkable similarities between epizootic IC and enzootic ID viruses have been documented. A total of 15 amino acid differences were identified between strains of both viruses isolated in Venezuela, and two of them were located within the E2 glycoprotein region, which is suspected to be the major determinant of equine virulence and amplification potential. Some studies have reported that a single amino acid substitution in the position E2-213 in ID Venezuelan strains (enzootic) resulted in a change in the equine viremia phenotype, generating high-titered viremia in horses and increasing the ability to infect the epidemic mosquito vector *Ae. taeniorhynchus* (Weaver et al. 2004, Aguilar et al. 2011, Weaver et al. 2012, Medina et al. 2015).

9.5.4.4 Enzootic Subtypes

Subtypes ID, IE, and IF and from II to VI have not been originally associated with epidemics or epizootics. They are called enzootic subtypes because they complete their cycle in wild habitats, involving mosquito vectors and rodent hosts (Fig. 9.3). These viruses are attenuated and unable to amplify in equines, but most of them can cause illness in humans. Only subtype IE has been related to an encephalitis outbreak in horses in Mexico, but equines were unable to amplify the virus. Because of this, equines may be dead-end hosts for these strains.

Enzootic strains are involved in active transmission cycles in tropical and subtropical areas of the Americas (Table 9.2). In endemic areas, mosquito isolates are mainly made from the mosquitoes *Culex* (*Melanoconion*) spp., which live in swampy areas and breed near aquatic plants. These mosquitoes feed on a variety of rodents, birds, and other vertebrates. However, isolations have also been performed from the genera *Aedes*, *Mansonia*, *Psorophora*, *Haemagogus*, *Sabethes*, *Deinocerites*, and *Anopheles*. Wild birds are susceptible to infection, but mammals (mainly rodents) are the most probable hosts, as shown by viral isolations, levels of viremia, serology, and disease resistance. Enzootic transmission cycles for VEEV ID, VEEV IE, EVEV, MUCV, and Tonate virus (Bijou Bridge virus) have been described; all of them – with the exception of Bijou Bridge – are maintained in cycles involving rodents and mosquitoes of the subgenus *Culex* (*Melanoconion*). In the western USA, Bijou Bridge virus is transmitted to birds by the bedbug *O. vicarious* (Aguilar et al. 2011, Weaver and Reisen 2010).

In Argentina, activity of Rio Negro (subtype VI) virus, which was isolated from *Cx.* (*Mel.*) *delpontei* and rodents of the genus *Akodon* in the subtropical area of Chaco and Formosa provinces, has been known for more than 3 decades. It is associated with the production of acute febrile disease and has been only recognized in this country (Contigiani et al. 1993, Cámara et al. 2003, Pisano et al. 2013, Pisano et al. 2012)

Pixuna (subtype IV) virus, first isolated from *Anopheles nimbus* mosquitoes in 1961 in northern

Brazil, has been also detected in Argentina (Pisano et al. 2010a, b). Enzootic circulation of multiple subtypes within a region (i.e., Argentina, Peru, and Venezuela) could result in the emergence of epizootic/epidemic strains. The interaction among strains and genetic mechanisms of adaptation to new hosts are postulated as mechanism for emergence of epizootic strains.

In Peru, epizootic/epidemic subtype IAB was recorded in the 1940s. Studies to determine the origin of this subtype led to the isolation of enzootic subtypes (ID and IIIC) from sentinel hamsters and mosquitoes (Aguilar et al. 2011).

In Brazil, the VEEV subtype IF circulates in the southeast and causes febrile illness and diarrhea in humans. It has also been isolated in the Amazon region (Calisher et al. 1982).

9.5.5 Control and Prevention

The neurotropic alphavirus should be continuously monitored through an active surveillance system that includes serological and virological surveillance (detection of antibodies and/or viral agent hosts and sentinel animals), clinical-epidemiological surveillance (by recording every disease suspected to be caused by an alphavirus), and entomological surveillance (mosquito collection, taxonomic identification, and identification of viral agent by RT-PCR and/or viral isolation).

When available, preventive measures should include the use of vaccines. Available vaccines are generally of two types: attenuated and inactivated virus vaccines.

For EEEV and WEEV, inactivated virus vaccines for veterinary use containing both viruses are prepared in Argentina. In the USA, a similar vaccine for veterinary and laboratory personnel use is prepared.

An attenuated virus vaccine for VEEV (TC83 strain) has been developed; it has been successful in immunizing horses and controlling epizootics in Venezuela. It is also used in the USA to protect laboratory personnel and military people. In humans, it can produce systemic reactions (fever, myalgias, leukopenia) so it is not used to immunize the general population. There is an inactivated virus vaccine at experimental level which is effective in humans and produces minimal side effects. There are attempts to develop a new vaccine against VEEV using recombinant techniques. Argentina is free from VEEV epizootic subtypes, so the use of this virus vaccine is not allowed, and vaccination is considered to be the cause of possible introduction (Go et al. 2014).

9.6 Polyarthritis Alphaviruses

The alphaviruses that cause joint disorders are chikungunya virus (Africa, Asia, Europe, America), o'nyong-nyong virus (central Africa), Ross River virus and Barmah Forest viruses (Australia and the Pacific Islands), Sindbis virus (cosmopolitan), and Mayaro virus (South America).

9.6.1 Pathogeny of Arthritogenic Alphaviruses

After subcutaneous inoculation through the bite of an infected mosquito in the skin, these alphaviruses disseminate in the host organism through the bloodstream. The liver, spleen, muscle, and lymph nodes are sites of primary replication, allowing an efficient virus spread. Langerhans cells facilitate virus delivery to the lymph nodes. Leukopenia in acute phase of the disease is a very common hematologic alteration in alphavirus infection, suggesting primary replication of the virus in the leukocytes. Interferon (IFN) program is early activated, but the alphaviruses developed several mechanisms to inhibit this antiviral response.

The acute phase of the disease involves virus replication followed by an inflammatory response in the target tissues, which is characterized by an extensive infiltration of lymphocytes (CD4+ and CD8+ T lymphocytes), NK cells, neutrophils, and macrophages (the main component). The increase in the levels of several proinflammatory cytokines and chemokines in the site of infection and in the plasma is associated with myositis and

arthralgia/arthritis. The secretion of metalloproteinases (MMPs) in the joint tissue may also contribute to articular damage. Persistence and severity of the symptoms may be related to the persistence of the virus or its products in the target cells with the subsequent accumulation of inflammatory mediators such as IL-6. A question that remains open is whether an autoimmune process is associated with the persistence of the inflammatory response, as observed for rheumatoid arthritis (Assunção-Miranda et al. 2013).

9.6.2 Treatment

There is no antiviral drug therapy available for these kinds of pathologies (polyarthritis of viral origin), with symptomatic treatment during acute phase of illness being only indicated. Patients are treated with nonsteroidal anti-inflammatory drugs, fluids, and medicines to relieve symptoms of fever and aching, such as ibuprofen, naproxen, acetaminophen, or paracetamol.

9.6.3 Sindbis Virus (WEEV Complex)

The Sindbis virus (SINV), with four subtypes, has been detected in Africa, Asia, Australia, and Europe. It was originally isolated from *Cx. univittatus* mosquitoes collected in Sindbis village, Nile Delta, Egypt, in 1952. SINV is maintained mainly between ornithophilic *Culex* spp. mosquitoes, but *Cs. morsitans*, *Coquillettidia richiardii*, *Mansonia africana*, *Aedes* spp., and *An. hyrcanus* are also involved. Birds act as hosts; rodents, amphibians, and bats have also been incriminated but less frequently. There are records of encephalitis cases in horses in South Africa. In humans it produces febrile illness with arthralgia and rash. Its geographic distribution includes Africa, Israel, Asian Turkey, India, Indonesia, Australia, New Zealand (Whataroa strain), China, Central Asia, Azerbaijan, Sweden, Finland, Russia, infrequently Italy (Sicily), Slovakia, and Germany (Assunção-Miranda et al. 2013, Hubálek et al. 2014).

9.6.4 Semliki Forest Complex

9.6.4.1 Mayaro Virus

The Mayaro virus (MAYV), with activity reported in the Americas, belongs to the Semliki Forest complex along with CHIKV, SFV, ONNV, and RRV (Old World viruses that produce the same clinical syndrome in humans) (Table 9.1).

This virus was first isolated from a sick man in Trinidad in 1954. It was subsequently isolated from humans with undifferentiated febrile illness in northern Brazil. It was also recovered from humans, primates, and wild mosquitoes in Bolivia, Brazil, Colombia, Venezuela, and Peru. Serological studies show that human infection is common in forested areas of northern South America. Activity has also been detected in Central America and human infections have recently been reported in Mexico. MAYV clinical cases in South America are sporadic and have been reported in individuals with history of recent activity in jungle areas. This is related to its *Haemagogus* mosquito vectors, which inhabit forested areas, preferably in the canopy. In recent years, travel-related infections by MAYV imported from South America to Europe and the USA have been increasingly reported.

At the molecular level, two MAYV genotypes can be identified: genotype D (of wide distribution) and L (of restricted distribution), which differ by more than 15 % in terms of their nucleic acid. However, that nucleotide divergence within each genotype did not exceed 5.9 % for genotype D and 3 % for genotype L. These data suggest that different patterns of transmission may be involved in the maintenance and evolution of MAYV.

The transmission cycle of MAYV could be similar to that of yellow fever virus, including monkeys as hosts and *Haemagogus* mosquitoes as vectors. Human outbreaks in Brazil in 1978 and 1991 contributed to the epidemiological knowledge of this virus. Antibodies were detected in a high percentage in *Callithrix* monkeys and viral isolations were made from *Haemagogus janthinomys* mosquitoes. It was further found that MAYV can infect and be

transmitted by *Ae. aegypti*, raising the possibility of urban outbreaks.

Demographic changes and the result of human activities (urbanization, deforestation, intensive farming) that produce dramatic changes in the environment may alter the frequency of clinical cases of this virus.

In humans, MAYV produces an acute febrile disease that can be confused with dengue. The clinical manifestation lasts between 3 and 5 days, and it is characterized by fever, headache, myalgia, rash, joint pain, and, less often, arthritis. No specific treatment or vaccine is available for MAYV infection. Studies aimed at having a live-attenuated vaccine are being developed (Assunção-Miranda et al. 2013, Muñoz and Navarro 2012, Weise et al. 2014).

9.6.4.2 UNA Virus

The epidemiology of UNAV is little known. It was first isolated from *Psorophora ferox* mosquitoes collected in the state of Pará, Brazil. It is widely distributed with low prevalence of infection in tropical and subtropical regions of Central and South America.

It is the only member of the Semliki Forest complex with documented activity in Argentina, where it was isolated in 1964 from a dead horse and a febrile one. In addition, antibodies against this virus were detected in birds and horses. Human infections were detected, suggesting they are contemporaneous to viral isolations. Another serological study in howler monkeys detected activity in Argentina and Paraguay, suggesting that this virus would be endemic to those regions and that these primates could be involved in the maintenance cycle. Similar epidemiological characteristics were described for MAYV.

UNAV strains, grouped into a single genotype, exhibit marked genetic diversity between them (about 28 %). UNAV and MAYV share 45 % of nucleotide identity. The genetic diversity pattern of UNAV suggests that this virus moved to new ecological niches, where it established maintenance enzootic foci following different evolutionary paths (Díaz et al. 2007, Powers et al. 2006).

9.6.4.3 Chikungunya Virus

The chikungunya virus (CHIKV) is an emerging virus that causes an acute febrile illness with arthralgia and rash of sudden onset. It belongs to the SFV complex, as UNAV and MAYV, in American continent (Table 9.1). The virus was first isolated from a feverish human in Tanzania in 1953. The disease by CHIKV is known as "chikungunya fever," "epidemic arthritis chikungunya," or just "chikungunya." This name from the language Makonde means "sickness of the folded walker" or "that which bends up." In India, it is known as "Aakyda," meaning "hard man." These names refer to the stooped posture as a result of the symptom of arthritis (Figueiredo and Figueiredo 2014, Caglioti et al. 2013).

- *Pathology in humans*. The incubation period in CHIKV infection is on average 2–4 days (range 1–12 days). The onset of illness is abrupt, with high fever, headache, back pain, myalgia, and arthralgia. Joint pains that occur in almost 80 % of patients can be severe and affect the extremities (ankles, wrists, phalanges), knees, elbows, etc., but this condition is rarely seen in children. Although this clinical manifestation is overcome in weeks or months, cases of persistence for several years and even chronic arthritis were observed. Joint pain and inflammation mainly affect symmetrically the small joints (such as those from fingers, wrists, and tarsus), but eventually occur in the large joints (such as those from knees and shoulders) and may also involve several joints simultaneously (polyarthralgia/polyarthritis). Besides rash and arthritis, myalgia is a very common symptom during alphavirus infection, also demonstrating the virus tropism for the muscular tissue.

Between 2 and 5 days after onset of symptoms, a maculopapular and erythematous rash mainly on the face, trunk, and limbs is displayed. These symptoms may remain until the tenth day after onset. Furthermore, it can cause conjunctivitis, pharyngitis, and lymphadenopathy. In children, localized petechiae and gingivorragia

can be observed. The peak incidence of serious infections was estimated in less than 0.02 %. The typical clinical presentations are myocarditis, central nervous system involvement, hemorrhagic manifestations, and possible teratogenic effects in neonatal CHIK.

On the other hand, there is no evidence that the lethality is produced by a direct action of CHIKV. The causes of severe clinical manifestations and deaths observed in recent virus epidemics in India and Reunion are unknown, but may be due to circulating strains of different virulence (Assunção-Miranda et al. 2013, Caglioti et al. 2013).

- *Epidemiology*. CHIKV is endemic to Africa, India, Southeast Asia, and the Philippines. During the period between 1960 and 1980, the virus was isolated repeatedly from different regions of Africa and Asia. Likewise, major epidemics occurred in India and the Philippines. In 2004, the virus emerged in the Indian Ocean islands (Lamu, Commodores, Seychelles, and others), where the vector *Ae. aegypti* is the most prevalent. Later, it spread to other areas where *Ae. albopictus* is the dominant vector (Reunion Island, Mauritius) and caused explosive epidemics with millions of cases in India after a silent period of more than 30 years. In 2006, imported cases by CHIKV were reported in different countries in Europe, with France and Italy having higher risk, due to the number of visitors coming from endemic regions, mainly India. In 2007, a native localized outbreak was reported for the first time in Europe, specifically in northeastern Italy, where *Ae. albopictus* was the vector. Between 2006 and 2011, imported cases were reported in the USA, Canada, Caribbean islands, Brazil, Oceania, and Australia associated with travelers from India and Indian Ocean islands (Coffey et al. 2014, Morrison 2014).

In late 2013 the Pan American Health Organization (PAHO)/World Health Organization (WHO) reported the first indigenous cases of CHIKV infection in the Caribbean islands (Guadeloupe, British Virgin Islands, Martinique, St. Barthélemy, and San Martin). In 2014, CHIKV local transmission has been identified in 41 countries and territories in the Caribbean, Central America, South America, and North America (Weaver 2014, Powers 2015).

The mosquitoes (*Ae. aegypti* and *Ae. albopictus*) that transmit the virus are found in a large part of the Americas, including areas of the USA. Since chikungunya virus is new to the Americas, most people in the region are not immune, meaning that they can be infected and the virus spread to other mosquitoes.

Genetic studies have identified three different lineages for CHIKV: Eastern, Central, and Southern African (ECSA), West African, and Asian genotypes. Recent isolates made in the Indian Ocean area form a distinct cluster within the large ECSA genotype. The genotypes should indicate independent evolutionary processes associated with isolated geographic regions. As expected in an RNA virus, there are differences among strains. In Central and West Africa, CHIKV is maintained in a sylvatic cycle between nonhuman primates and forest mosquitoes *Aedes* spp., from which it was repeatedly isolated. Wild mosquito species primarily involved in the sylvatic cycle (depending on ecological and geographical conditions) are *Ae. furcifer*, *Ae. taylori*, *Ae. luteocephalus*, *Ae. africanus*, and *Ae. neoafricanus*; epidemic vectors would involve *Ae. furcifer* and *Ae. taylori. Aedes aegypti* was associated as vector in urban epidemics from East Africa and the Indian Ocean.

The mosquito *Ae. albopictus* is strongly associated with humans but also with peridomestic habitats and therefore responsible for the large urban epidemics in Reunion, India, and Southeast Asia. In Asia, *Ae. aegypti* was also incriminated as the main vector. Besides these mosquito vector species, other peridomestic mosquitoes which have been detected in abundance include *Ae. albopictus*, *Ae. vittatus*, and *An. stephensi*.

Little is known about vertebrates that may be involved in maintenance cycles. In urban populations, epidemic periods, the virus is maintained

by the mosquito-human-mosquito cycle. In non-epidemic periods, reservoirs may be nonhuman primates or other unidentified vertebrates. The epizootic in monkeys occurs when immunity at populational level is very low. These animals develop viremia but have no serious clinical manifestations (Coffey et al. 2014, Morrison 2014, Caglioti et al. 2013).

- *Control and prevention*. There is no vaccine available to prevent chikungunya fever. An experimental live-attenuated virus vaccine (TSI-GSD-218) obtained from a strain isolated from a patient with CHIKV from Thailand and attenuated by serial passages in MRC-5 cells was tested in human volunteers. This vaccine demonstrated to be a good inductor of neutralizing antibody response that endures overtime. Effective preventive measures consist of individual protection against mosquito bites and vector control of *Ae. aegypti* and *Ae. albopictus*. Sick people should be kept protected from potential mosquito bites, either by staying in the home or using insecticide or repellent during the first 4–6 days after infection, to avoid infections by new vectors and consequent amplification of the disease.

Other arthritogenic alphaviruses are ONNV, RRV (Semliki Forest virus complex), SINV (WEEV complex), and BFV (Barmah Forest virus).

9.6.4.4 O'Nyong-Nyong Virus (ONNV)

ONNV was detected for the first time in Uganda (Africa) in 1959, during a major central African epidemic of ONN fever that began in northern Uganda and spread to Kenya and Tanzania (1959–1962). It is genetically and serologically related to CHIKV and is restricted to the African continent. Typical clinical features include low-grade fever, symmetrical polyarthralgia, lymphadenopathy (particularly of the posterior cervical region), and a generalized papular or maculopapular exanthem. Although some patients suffered from prolonged joint pain during the recovery phase, no fatal cases or permanent sequelae were observed. During 1996 and 1997, south-central Uganda experienced the second ONN fever epidemic ever recognized.

Anopheline mosquitoes (*An. funestus*) were involved as the primary epidemic vectors. Vertebrate studies conducted during the epidemic failed to incriminate rodents or mongooses as amplifying hosts of the virus.

A laboratory-confirmed case of an ONNV infection imported into Europe was recently reported. This patient most likely was infected in the eastern part of Kenya (Kisumu region). Because of the serological and clinical similarities of ONNV and CHIKV infections, infections in travelers may have been wrongly diagnosed as CHIK. Similarly to other arboviruses, especially CHIKV and dengue viruses, ONNV might have the potential to spread to areas outside Africa. There are no known invasive anopheline vectors for ONNV in Europe, but it was demonstrated that the species *Ae. aegypti*, found in some parts of Europe, might be a competent vector for ONNV (Assunção-Miranda et al. 2013).

9.6.4.5 Ross River Virus (RRV)

Ross River virus (RRV) is endemic and enzootic in Australia and Oceania (Papua New Guinea, the Solomon Islands, and the South Pacific islands) and Southeast Asia, where it caused major epidemics. It was first isolated from *Ae. vigilax* mosquitoes collected near Townsville in northern Queensland in 1959. In Australia, RRV disease is the most widely spread mosquito-borne disease. In 2011, RRV infection accounted for 63 % (5149 cases) of all mosquito-borne disease records. This virus causes a nonfatal, but prolonged and debilitating disease known as epidemic polyarthritis or RRV disease. The incubation period may be as long as 21 days or as short as 3 days (usually 7–9 days).

The epidemiology of RRV disease is complex because transmission cycles are driven by various mosquito species and vertebrate hosts within a variety of disparate geoclimatic regions. The natural vertebrate hosts include marsupial animals (e.g., kangaroos, wallabies) and possibly other animals (e.g., dogs, cats, horses, possums). More than 30 mosquito species have been implicated as vectors of RRV, with *Ae. vigilax* and *Ae. camptorhynchus*

being significant in the coastal regions and *Cx. annulirostris* being common in tropical areas and temperate regions that are subject to flooding or irrigation during summer. Species such as *Ae. notoscriptus* may be important in semirural and urban areas (Tong et al. 2008).

9.6.4.6 Barmah Forest Virus (BFV) (BFV Complex)

Barmah Forest virus (BFV) was first isolated from a pool of *Cx. annulirostris* mosquitoes collected in southeastern Australia in 1974. Although the animal reservoir remains unknown, native animals, such as kangaroos, brushtails, and wallabies, could be involved in the cycle of infection. The mosquitoes that can spread the virus are *Ae. vigilax*, *Cx. annulirostris*, *Ae. normanensis*, and *Ae. notoscriptus*. Vector competence studies showed that *Ae. notoscriptus* could act as an efficient vector of BF in urban environments. BFV infection cannot be spread from person to person. Increases in travel and trade will undoubtedly increase the risk of BFV introduction to other continents. At present, BFV has been the cause of outbreaks of human disease in Australia (Jacups et al. 2008).

9.7 Other Alphaviruses Pathogenic for Animals

9.7.1 Getah Virus (GETV) (SFV Complex)

GET virus was first isolated from *Cx. gelidus* mosquitoes near Kuala Lumpur (Malaysia) in 1955. Disease in animals (horses) was first recognized in Japan, 1978. GETV is widely distributed in the countries of Southeast Asia and in northern Australia along the Pacific Ocean. The mosquito species implicated as vectors are *Cx. gelidus*, *Cx. tritaeniorhynchus*, *Cx. fuscocephala*, *Ae. vexans nipponensis*, *Ae. nigripes*, *Ae. communis*, and *Ae. excrucians*. Horses, pigs, and wild boars would act as vertebrate hosts. The virus is known to be pathogenic for horses (often racehorses) and pigs. In horses, the disease is characterized by depression, anorexia, fever, nasal discharge, urticarial rash, edema of the hind limbs, swelling of the submandibular lymph nodes, and lymphocytopenia (experimentally confirmed). It can also cause abortions in pigs. The virus (very closely related to Ross River virus) has not been linked to illness in humans; however, neutralizing antibodies to GETV have been identified in human sera and in birds in Malaysia, in northern Australia, and in China. Outbreaks of GETV infection were first recorded in racehorses at two training centers in Japan in 1978. Since then, several outbreaks of the disease have been reported in Japan especially, in horse racing tracks (1991–1997), and one was reported from India in 1990 (Hubálek et al. 2014).

9.8 Concluding Remarks

Pathogenic alphaviruses for humans can produce very different clinical manifestations. The same virus can cause symptoms not previously recorded as a result of changes at the genomic level that cause changes in its virulence and disease with different clinical characteristics.

The co-circulation of multiple viral species belonging to the same serological complex in a particular geographic region could favor the emergence of new pathogenic variants for humans and animals of veterinary importance.

The biological characteristics of transmission and maintenance of these viruses make their infections independent of socioeconomic development of a country or region. In addition, its spread is favored by increasing circulation by tourism or commerce.

The current scenarios of emergence and reemergence observed worldwide indicate the need to intensify surveillance systems through monitoring of vectors and hosts, as well as deeper understanding of the biology, epidemiology, and pathogenesis of these zoonoses. Finally, the knowledge of natural genetic variability of circulating strains in each region will lead to improved diagnosis, treatment, and prevention of these diseases.

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