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Review

Zoonotic mosquito-borne flaviviruses: Worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases

H. Weissenböck [a,*], Z. Hubálek [b], T. Bakonyi [c,d], N. Nowotny [d]

[a] Institute of Pathology and Forensic Veterinary Medicine, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria
[b] Medical Zoology Laboratory, Institute of Vertebrate Biology, Academy of Sciences, Valtice, Czech Republic
[c] Department of Microbiology and Infectious Diseases, Faculty of Veterinary Science, Szent István University, Budapest, Hungary
[d] Zoonoses and Emerging Infections Group, Clinical Virology, Department of Pathobiology, University of Veterinary Medicine, Vienna, Austria

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ABSTRACT

An update on the mosquito-borne flavivirus species including certain subtypes, as listed in the Eighth Report of the International Committee on Taxonomy of Viruses, is given. Special emphasis is placed on viruses which have been shown to cause diseases in animals, and viruses for which no pathogenicity has been proven yet. Several recent examples (Usutu virus and lineage-2 West Nile virus in central Europe, Zika virus in Micronesia) have shown that sources providing information on such scientifically largely neglected viruses are valuable tools for scientists and public health officials having to deal with such disease emergences. Furthermore the effects of global warming will lead to introduction of competent mosquito vectors into temperate climate zones and will increase efficiency of viral replication in less competent vector species. This, facilitated by rising global travel and trade activities, will facilitate introduction and permanent establishment of mosquito-borne viruses, some of which may become of public health or veterinary concern, into novel environments, e.g. industrialized countries worldwide.

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1. Introduction

A zoonosis is any disease or infection that is naturally transmissible from vertebrate animals to humans (World Health Organization, 2008). The formerly used terms anthropozoonosis (disease transmissible from human beings to animals) and zooanthroponosis (disease transmissible from animals to humans) have been abandoned, because they were frequently not properly used and created more confusion than clarity in the past (Hubálek, 2003). The definition of "zoonosis" does not state that the transmission of the infection must be direct. Thus transmission of an infectious agent from a vertebrate host

* Corresponding author. Tel.: +43 125077 2418; fax: +43 125077 2490.
E-mail address: Herbert.Weissenboeck@vetmeduni.ac.at (H. Weissenböck).

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population via vectors (like mosquitoes) to humans fulfils the criteria of being considered a zoonosis (sometimes the arthropod-borne diseases are also called "metazoonoses"). Occasionally the term emerging zoonosis is used, when the disease has appeared in a population for the first time, or may have existed previously but is rapidly increasing in incidence or geographic range (Morse, 1995).

In recent years veterinary and medical scientists recognized a number of unexpected emergences of flaviviral zoonoses worldwide. The introduction of West Nile virus (WNV) into the New World, the emergence of Japanese encephalitis virus (JEV) in Australia and of Usutu virus (USUV) in central Europe are just a few prominent examples.

As this review is written for a veterinary journal, we will especially focus on flaviviral infections which do not only involve vertebrate animals as hosts (which, except for dengue and urban yellow fever, may be the case for all mosquito-borne flaviviruses), but also are pathogenic for domestic or wild animals. Furthermore, we feel that information concerning the most prominent and medically important mosquito-borne flaviviruses, i.e. dengue viruses (DENVs), yellow fever virus (YFV), JEV and WNV is readily available and a number of excellent review articles and book contributions have been published. Thus, these viruses will not receive extensive attention in this review. However, we intend to review current knowledge on all mosquito-borne flaviviruses, a large number of which has so far never or only sporadically been involved in animal or human disease. Many of these viruses are largely scientifically neglected. The example of USUV emergence in Europe, however, clearly indicates that viruses considered non-pathogenic in their natural habitat can gain unforeseeable pathogenicity in other geographic areas (Chvala et al., 2007).

2. History

Long before the isolation of the causative agents and the elucidation of transmission cycles involving arthropods, historic documents report disease outbreaks compatible with dengue or yellow fever especially in the Caribbean but also in Europe particularly in areas surrounding harbours (Fontenille et al., 2007). Even decades before the first isolations of the causative viruses the role of mosquitoes in the transmission of yellow fever and dengue had been revealed and subsequent mosquito control programs, especially aiming at eliminating *Aedes* (*Stegomyia*) *aegypti*, the vector of urban yellow fever and dengue, led to significant decline of human infections and deaths. In Rio de Janeiro, the number of yellow fever deaths was reduced from two thousand in 1903 to zero in 1909 (Figueiredo, 2000). The first isolations of mosquito-borne flaviviruses took place between the late 1910s and early 1940s: 1918: Murray Valley encephalitis virus (MVEV) in Australia; 1927: YFV in Ghana; 1933: St. Louis encephalitis virus (SLEV) in USA; 1934: JEV in Japan; 1937: WNV in Uganda; 1943: DENV-1 serotype Hawaii (Theiler and Downs, 1973; Mackenzie and Broom, 1995; Endy and Nisalak, 2002). The first vaccine against a mosquito-borne flavivirus was a yellow fever vaccine which was developed by Theiler in 1937 and first successfully applied in 1938 (Figueiredo, 2000). Scientifically documented major outbreaks of mosquito-borne flaviviral diseases in human beings are numerous: the most severe epidemic ever of yellow fever in Africa with estimated 100,000 cases and 30,000 deaths occurred in south-western Ethiopia beginning in late 1960 and continuing into the following years (Theiler and Downs, 1973). Between 1927 and 1928, a large epidemic of dengue occurred in Athens, Greece. It has been assumed that 90% of the population in Athens and Piraeus had been attacked, resulting in one million cases and more than 1000 deaths (Gubler, 1997). The first documented large Japanese encephalitis epidemic occurred in 1924 in Japan involving over 6000 cases (Endy and Nisalak, 2002). First documented animal mortalities due to mosquito-borne flaviviruses were howler monkeys due to yellow fever in Trinidad, 1954 (Anderson and Downs, 1955), neonatal and aborted lambs in South Africa due to Wesselsbron disease virus (WESSV), 1955 (Swanepoel and Coetzer, 2004), horses in Egypt, 1959 and later, 1962, in Southern France (Murgue et al., 2002) due to WNV, and turkeys due to Israel turkey meningoencephalitis virus (ITV) in Israel, 1960 (Guy and Malkinson, 2008).

3. The infectious agents and associated diseases in animals and humans

The International Committee on Taxonomy of Viruses (ICTV) assigns mosquito-borne flaviviruses to seven groups (Table 1), based on genetic and antigenic relationships. Currently there are 39 defined members belonging to the mosquito-borne viruses (Thiel et al., 2005) of the genus *Flavivirus*. They include the globally most important pathogens of this genus, such as YFV, DENVs, JEV und WNV. They are small, enveloped viruses that contain a single-stranded positive-sense RNA genome, approximately 11 kb in length. A single open reading frame encodes three structural proteins, capsid (C), premembrane/membrane (PrM/M) and envelope (E), and seven non-structural proteins, NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5. The polyprotein is co-translationally and post-translationally cleaved by host proteases and the viral serine protease NS2B/NS3. The capsid protein is associated with the viral RNA forming the nucleocapsid, while the viral envelope contains the prM/M and the E proteins. The E protein is a major flavivirus antigenic determinant and is involved in attachment and entry of the virion into the cell. The prM protein is essential for proper folding of the E protein and is cleaved to M by furin prior to release of the mature virion from the cell (Lindenbach and Rice, 2003). The functions of all non-structural proteins are only partly understood, but have been shown to contribute essential functions to the various stages of viral replication. NS5 is the largest and most highly conserved flavivirus protein and serves as RNA-dependent RNA polymerase (McMinn, 1997).

Mosquito-borne flaviviruses usually infect a variety of vertebrate and mosquito species. Some have a limited host and vector range (e.g. YFV), others replicate in a large number of vectors and hosts (e.g. WNV). Mosquito-borne flaviviruses are found on all continents except Antarctica. Some have an extremely widespread distribution (e.g.

Table 1
List of mosquito-borne flaviviruses (genus *Flavivirus*).

Virus (italics: virus species, roman: virus subtypes or strains)	Abbreviation	Numbers citations	Geographic distribution	Human disease	Animal disease	Full sequences available
Aroa virus group						
Aroa virus						
Aroa virus[a]	AROAV	0	Venezuela	Unknown	Unknown	n
Bussuquara virus	BSQV	4	Brazil, Colombia, Panama	1 case: fever, joint pain, headache	Unknown	y
Iguape virus	IGUV	1	Brazil	Unknown	Unknown	y
Naranjal virus	NJLV	0	Ecuador	Unknown	Unknown	n
Dengue virus group						
Dengue virus		4386	Tropics, subtropics	Fever, rash, vasculopathy	Unknown (monkeys?)	
Dengue virus 1	DENV-1					y
Dengue virus 2	DENV-2					y
Dengue virus 3	DENV-3					y
Dengue virus 4	DENV-4					y
Kedougou virus						
Kedougou virus	KEDV	3	Senegal, CAR	Unknown	Unknown	y
Japanese encephalitis virus group						
Cacipacore virus[a]						
Cacipacore virus[a]	CPCV	3	Brazil	Unknown	Unknown	n
Japanese encephalitis virus						
Japanese encephalitis virus	JEV	2140	Asia	Thousands of cases: encephalitis	Pigs, horses	y
Koutango virus[a]						
Koutango virus[a]	KOUV	7	Senegal	3 cases: fever, rash	Unknown	n
Murray Valley encephalitis virus						
Alfuy virus	ALFV	6	Australia	Unknown	Unknown	y
Murray Valley encephalitis virus	MVEV	134	Australia, Papua New Guinea	Tens of cases: fever, rash, encephalitis	Young sheep and monkeys: encephalitis	y
St. Louis encephalitis virus						
St. Louis encephalitis virus	SLEV	256	Americas	>2500 cases: fever, encephalitis	Unknown	y
Usutu virus						
Usutu virus	USUV	19	Africa, Europe	2 cases: fever, rash	Different bird species	y
West Nile virus						
Kunjin virus	KUNV	175	Australia, Indonesia, Malaysia	Fever, rash, lymphadenopathy	Unknown	y
West Nile virus	WNV	3299	Worldwide	Thousands of cases: encephalitis	Reptiles, birds, mammals	y
Yaounde virus						
Yaounde virus	YAOV	2	Central Africa	Unknown	Unknown	n
Kokobera virus group						
Kokobera virus						
Kokobera virus	KOKV	10	Australia, Papua New Guinea	Acute polyarthritis	Unknown	y
Stratford virus	STRV	3	Australia	Unknown	Unknown	n
Ntaya virus group						
Bagaza virus						
Bagaza virus	BAGV	5	Africa	Unknown	Unknown	y
Ilheus virus						
Ilheus virus	ILHV	15	South and Central America	Sporadic: fever, headache, myalgia, CNS symptoms	Unknown	y

Table 1 (*Continued*)

Virus (italics: virus species, roman: virus subtypes or strains)	Abbreviation	Numbers citations	Geographic distribution	Human disease	Animal disease	Full sequences available
Rocio virus	ROCV	20	Brazil	Several epidemics (>1000 cases): encephalitis	Unknown	y
Israel turkey meningo-encephalomyelitis virus						
Israel turkey meningoencephalo-myelitis virus	ITV	10	Israel; South Africa	Unknown	Turkeys	n
Ntaya virus						
Ntaya virus	NTAV	1	Africa	Unknown	Unknown	n
Tembusu virus						
Tembusu virus	TMUV	5	Malaysia, Thailand	Unknown	Unknown	n
Spondweni virus group						
Zika virus						
Spondweni virus	SPOV	1	Africa (southern and central)	5 cases: fever, myalgia, rash	Unknown	n
Zika virus	ZIKV	18	Africa, Asia, Micronesia	Sporadic cases, 1 epidemic: fever, headache, rash	Unknown	y
Yellow fever virus group						
Banzi virus						
Banzi virus	BANV	25	South Africa	2 cases: fever	Unknown	n
Bouboui virus						
Bouboui virus	BOUV	1	Africa	Unknown	Unknown	n
Edge Hill virus						
Edge Hill virus	EHV	5	Australia	1 case: myalgia, arthritis	Unknown	n
Jugra virus						
Jugra virus	JUGV	0	Malaysia	Unknown	Unknown	n
Saboya virus[a]						
Potiskum virus[a]	POTV	3	Africa	Unknown	Experimental fatal disease: chicken	n
Saboya virus[a]	SABV	1	Africa	Unknown	Unknown	n
Sepik virus						
Sepik virus	SEPV	3	New Guinea	1 case: fever, headache	Unknown	y
Uganda S virus						
Uganda S virus	UGSV	3	Africa	Unknown	Unknown	n
Wesselsbron virus						
Wesselsbron virus	WESSV	28	Africa, Madagascar, Thailand	9 cases: fever, myalgia, arthralgia, CNS signs, hepato- and splenomegaly	Sheep, goat, cattle	n
Yellow fever virus						
Yellow fever virus	YFV	1013	Tropical Africa and South America	Thousands of cases: pantropic	Monkeys	y

Order, group assignments and abbreviations according to ICTV, 2005. The number of citations should reflect the degree of scientific coverage of the respective viruses. Data are extracted from the Scopus database (www.scopus.com) and only citations including the entire virus names were counted (accessed 30 July, 2008). Further, information on geographical distribution, occurrence of human disease and major clinical signs, as well as occurrence of animal disease is given. The last column summarized whether full sequences of the respective virus are deposited in GenBank database (http://www.ncbi.nlm.nih.gov/sites/entrez) (accessed 25 September, 2008). n: full sequence not available, y: full sequence available.

[a] These viruses have not (yet) been isolated from mosquitoes.

WNV), others are restricted to endemic areas (e.g. MVEV, ITV, Ilheus virus [ILHV]).

The numerous species of mosquito-borne flaviviruses are characterized by strongly different pathogenicities. Some are responsible for thousands of human fatalities worldwide (YFV, DENV, JEV), others have not been associated with any human or animal diseases so far (e.g. Kedougou virus [KEDV], Cacipacore virus [CPCV], Yaounde virus [YAOV]). First of all, the potential to cause disease in humans is of interest, but secondly, also the ability to induce losses in livestock or wild animals is of economic and ecological importance. Since this review is written for a veterinary journal, viruses with proven pathogenicity for animals are summarized in Table 2. Much effort has been invested into elucidating molecular determinants of flavivirus virulence. Most of this work has been done on viruses pathogenic for humans, like YFV, DENV, JEV, and more recently WNV is being increasingly studied. Based on natural strains of viruses, which have been selected by serial passaging, plaque purification or neutralization escape, and showed altered (usually attenuated) virulence for mice, a number of potential amino acid changes have been identified which may be responsible for this altered biological behaviour. The majority of such amino acid changes have been studied on the E protein, which plays a major role in flavivirus attachment to host cells and membrane fusion with target cells. Single natural mutations either increasing or attenuating natural virus strains have been engineered by targeted site mutagenesis and have been introduced into virus strains of high or low virulence in order to study the effect of a single altered amino acid on the virulence. The virulence of viruses is mostly studied by experimental mouse inoculation. Especially two indicators are investigated either by intracerebral or peripheral (usually subcutaneous) infection: "neurovirulence", which is the ability of a virus to initiate cytopathic infection in the central nervous system (CNS) and to cause encephalitis, and "neuroinvasiveness", which is the ability of a virus to replicate in peripheral tissues, induce viraemia and invade the CNS (McMinn, 1997). A number of molecular determinants of neuroinvasiveness and neurovirulence have been identified (reviewed in: Lee and Lobigs, 2000; Hurrelbrink and McMinn, 2003). Motives located on the E protein, which have been implicated with viral virulence across species boundaries, are the integrin binding RGD (388–390) sequence (Lee and Lobigs, 2002; Wicker et al., 2006) and the *N*-linked glycosylation motif NYS (154–156). Substitutions of the Asp_{390} residue for Gly or His resulted in a strongly attenuated virus phenotype in MVE. Intriguingly, the RGD motif is not found in the E protein of all flaviviruses. It is found in all members of the Japanese encephalitis group, but not in DENVs. There is also a lineage 2 WNV strain (Hungary04) (Bakonyi et al., 2006) with proven pathogenicity to birds and horses which has a substitution (D390E) within this motif. Substitutions at residues 154 or 156 lead to loss of protein E glycosylation, which results in attenuation of neuroinvasiveness. Apart from the E protein, also substitutions at non-structural proteins, such as NS4B or NS3 have been associated with virulence changes (Wicker et al., 2006). However, defining

Table 2
Mosquito-borne flaviviruses with proven potential to cause spontaneous diseases in wild and domestic animals.

Virus species	Affected animal species	Major clinical signs	Pathological lesions	Geographical distribution	Disease frequency	References
JEV	Pigs, horses	Pigs: Abortus Horses: neurological signs	Pigs: none Horses: encephalitis	South-east Asia	Extremely rare	Endy and Nisalak (2002) and Yamada et al. (2004)
USUV	Several wild bird species	Weakness; sudden deaths	Encephalitis, Necroses and inflammation in many organs	Central Europe	Several epizootics, otherwise infrequent	Chvala et al. (2007) and Bakonyi et al. (2007)
WNV	Numerous bird and mammal species (esp. horses), alligators	Birds: sudden deaths, nerval signs Mammals: neurological signs	Mammals and birds: encephalomyelitis; Birds: necroses and inflammation in many organs	Worldwide	North America: frequent; other parts of the world: occasional epizootics, otherwise infrequent	Komar (2003), Koopmans et al. (2007) and Bakonyi et al. (2006)
ITV (syn: BAGV)	Turkeys	Paresis and paralysis	Encephalitis	Israel, South Africa	Occasional outbreaks (in Israel control by vaccination)	Guy and Malkinson (2008)
WESSV	Cattle, sheep, goats	Abortion, neonatal morbidity and death	Hepato- and splenomegaly, liver necroses	Africa, Thailand	Irregular outbreaks	Swanepoel and Coetzer (2004)
YFV	Monkeys	Fatalities	Liver necroses	South America, Africa	Widely unknown, probably underestimated	Barrett and Monath (2003)

molecular determinants of virulence is not a straightforward task. Numerous factors in the highly complex chain of events during flaviviral replication affect replication efficiency, both from pathogen and host. Certainly there is no single indicator of pathogenicity or virulence, but more likely a coordinated interplay of several determinants. Also, virulence in the mouse model does not necessarily reflect the degree of virulence for other species, including humans. Recently, the search for virulence markers has been extended to natural hosts. Brault et al. (2007) have shown that a single substitution at NS3 residue 249 confers increased pathogenicity of WNV for American crows, a fact which has been responsible for the dramatic losses in this particular bird species during the WNV epidemic in North America. All lineage 1 WNV strains, which proved pathogenic for birds so far (NY 1999, Israel 1998, Egypt 1951, Hungary 2003), had this particular substitution, while lineage 1 WNV strains, which are non-pathogenic for birds, did not show this substitution.

In the following paragraphs, supported by Table 1, a short overview of all mosquito-borne flaviviruses, grouped according to the suggestion of the ICTV, is given. Although sub-species groupings are not considered as taxonomical units of the ICTV, certain important subtypes, lineages, strains and isolates are also indicated. We decided to include all viruses, also those of which no pathogenicity for vertebrates has been reported so far, because some of these might become pathogenic in the future. This possibility has been shown by the emergence of a strain of USUV in Europe, which was highly pathogenic for certain bird species, or with Zika virus causing a recent epidemic in Micronesia (Lanciotti et al., 2008).

The Aroa virus group includes one virus species, *Aroa virus*, which is divided into four subtypes, Aroa virus, Bussuquara virus, Iguape virus, Naranjal virus. All these viruses have only been found in South America so far. There is very little information available on these viruses. Aroa virus (AROAV) has been isolated from a sentinel hamster in Venezuela (but not yet from mosquitoes), Iguape virus (IGUV) from a sentinel mouse in Brazil, and Naranjal virus (NJLV) from a sentinel hamster and from mosquitoes in Ecuador. These three viruses have not been linked to diseases of vertebrates. Bussuquara virus (BSQV) has been isolated from sentinel and wild rodents and from mosquitoes in Brazil, Colombia and Panama, and has been associated with a single case of febrile disease with arthralgia in a human (Karabatsos, 1985; Figueiredo, 2000; Calisher and Gould, 2003; Shope, 2003).

The Dengue virus group comprises the *Dengue viruses 1–4* and *Kedougou virus*, which – according to Kuno and Chang (2007) – has a too low level of genetic relatedness to DENVs to justify placement in this group. The four DENVs cause more human infections annually than any other flavivirus (at least 50 millions with vasculopathy in 1–10% of the most severe cases). It seems to be the only mosquito-borne flavivirus for which human beings serve as the major host species and for which there is no continuous animal reservoir (however, some primates might act as hosts for dengue viruses in south-east Asia and tropical Africa). Primary vector is *Ae.* (*St.*) *aegypti* but *Ae. albopictus* (*Stegomyia albopicta*) has also been proven to be a competent vector (Fontenille et al., 2007; Gould and Solomon, 2008). KEDV has only been isolated from *Aedes* sp. in Africa (Senegal, Central African Republic), and there are no reports of associated disease in vertebrates (Calisher and Gould, 2003; Adam and Digoutte, 2005).

The Japanese encephalitis virus group includes eight virus species: Cacipacore virus, Japanese encephalitis virus, Koutango virus, Murray valley encephalitis virus, St. Louis encephalitis virus, Usutu virus, West Nile virus, and Yaounde virus. CPCV was isolated from a black-faced antthrush (*Formicarius analis*) in Brazil, and has never been isolated from mosquitoes or associated with disease in vertebrates (Calisher and Gould, 2003). JEV is another important human pathogen of the mosquito-borne flaviviruses. Up to 50,000 cases of encephalitis, with a fatality rate of approximately 25% occur each year. The natural distribution range of JEV is south-east Asia and Australasia. The vectors are *Culex* spp., predominantly *Cx. tritaeniorhynchus*. Virus activity is naturally maintained through bird-mosquito cycles and pigs are important amplifying hosts. Natural infection in pigs is generally inapparent, except for occasional stillbirths and abortions when pregnant sows are infected. Experimentally, using extremely high virus amounts, nonsuppurative encephalitis in piglets could be induced (Yamada et al., 2004). When JE was still widespread in Japan, epizootics of encephalitis in horses tended to coincide with human epidemics, but nowadays equine disease has become rare (Halstead and Jacobson, 2003). Detailed information on JEV is available from numerous sources. Koutango virus (KOUV) was for the first time recovered from an African rodent (*Tatera kempi*) in Senegal. So far, this virus has only been isolated from rodents or ixodid ticks, and there is a single reported human infection (fever, rash) in a laboratory worker (Adam and Digoutte, 2005).

MVEV has been found in Australia and Papua New Guinea. Wading birds are considered the most important natural reservoir hosts. The freshwater mosquito *Cx. annulirostris* is the principal epidemic vector. The last major epidemic in Australia with 58 human cases of encephalitis (fatality rate 20%) occurred in 1974. Since then, only sporadic cases (between one and 15 annually) have been reported. Disease in animals caused by MVEV is unknown (Russell and Dwyer, 2000). According to ICTV, Alfuy virus (ALFV) is classified as a subtype of WVEV. Recent data, however, suggest reclassification of ALFV as a distinct virus in the JEV group (May et al., 2006). It was originally isolated from a swamp pheasant (*Centropus phasianius*) and is regularly recovered from *Culex* mosquitoes in Australia. There are no confirmed diseases in vertebrates caused by this virus (May et al., 2006). SLEV is distributed over most of the American continent. Birds of the orders Passeriformes and Columbiformes seem to be the major vertebrate hosts and mosquitoes within the genus *Culex* are the principal vectors. During the last 75 years a number of epidemics involving hundreds of cases have been reported from a number of southern and mid-western US states. In total, more than 10,000 severe cases with more than 1000 deaths are estimated to have occurred between 1933 and 2000. The last major epidemic with 222 laboratory-confirmed human cases and 11 deaths

was recorded in Florida in 1990. Endemic St. Louis encephalitis affects an average of 25 individuals per year in the US. Infected wild birds, as well as mammals do not show clinical illness (Reisen, 2003).

USUV was originally isolated from *Culex univittatus* in 1959 in South Africa. In the following decades, additional strains of USUV were recovered from different mosquito and bird species in several African states (Adam and Digoutte, 2005). There is a single report of a human infection presenting as fever and rash. Until 2001, USUV has never been associated with severe disease or mortality in any vertebrate species. In 2001, an obviously more virulent strain of USUV emerged in central Europe, causing considerable avian mortality, especially among blackbirds (Turdus merula), but also some other bird species, between 2001 and 2003 (Weissenböck et al., 2002; Bakonyi et al., 2004; Chvala et al., 2007). This strain of USUV has now become endemic in central Europe and its presence has been verified in Austria, Hungary, Switzerland and Italy. Wild bird mortality has waned and is currently only sporadically seen in endemic areas. Instead, a high level of seropositivity of wild birds in such areas has been recognized (Meister et al., 2008). In Europe, *Culex* mosquitoes have been considered to serve as principal vectors and many bird species are natural hosts. According to serological data infections of human beings do not occur infrequently and in some individuals transient rash seems to be associated with the infection. Serological data of birds from the UK, but also from Germany, Poland, the Czech Republic and Italy indicate occasional presence or even circulation of USUV in these countries (Linke et al., 2007; Hubálek et al., 2008). In Spain a different, obviously less virulent strain of USUV has been detected in *Culex* mosquitoes, which was not associated with any avian mortality (Busquets et al., 2008).

WNV has the most widespread geographical distribution and the largest vector and host range of all mosquito-borne flaviviruses. Its first isolation dates back to 1937 (see Section 2). Compared to the highly pathogenic viruses DENV, YFV, and JEV, the pathogenicity of this virus for humans has been considered comparatively low. However, more virulent genotypes have started to emerge from 1996 onwards, leading to epidemics in Romania, Israel, Russia, and peaked in the introduction of the virus to the New World leading to unparalleled morbidity and mortality rate caused by this virus. A number of excellent and detailed reviews on the WNV epidemic in the Americas are available, so this issue will not be the focus of this paper. Alarmed by the rapid spread of the virus in the New World, also surveillance efforts in other parts of the globe, including Europe, were intensified and led to a number of novel insights concerning ecology and epidemiology of this virus. Serological surveys showed that a certain (generally low) degree of WNV activity is present all over Europe. Human and animal affections, however, are comparatively rare. Recent autochthonous human infections, all of them rather harmless cases of fever and headache, have been reported from Spain, Portugal, the Czech Republic and Hungary. Among the mosquito-borne flaviviruses, WNV is also the most important veterinary pathogen. Its pathogenicity for horses, where it induces nonsuppurative encephalomyelitis, has been well known for a long time. While in the US thousands of horses succumbed to this infection, in Europe equine cases were concentrated in a few clusters (Murgue et al., 2002). Also a number of other domestic and wild mammals have been shown to sporadically develop severe encephalitic disease. Furthermore, WNV has been shown to be highly pathogenic for a large number of wild and domestic bird species (reviewed in: Komar, 2003). WNV-associated bird mortality occurred (and still occurs) on a large scale in North America, but several episodes, affecting geese or birds of prey, have also been noticed elsewhere (Israel, Spain, Hungary: Bakonyi et al., 2006; Koopmans et al., 2007; Höfle et al., 2008). Currently, the European country where the most conspicuous virus activity has been recorded is Hungary. Here two lineages of WNV are coexisting, and disease was found in wild and domestic birds as well as in sheep and horses. The lineage 2 virus seems to be endemic, as genetically very closely related viruses have been found within five years. Further, the lineage 2 strain present in Hungary is remarkable for its pathogenicity for animals (birds and mammals).

YAOV has been isolated from mosquitoes, a bird, and a few rodents in several African states. There is no information on possible pathogenicity for animals or humans (Mackenzie et al., 2002; Adam and Digoutte, 2005).

The Kokobera virus group includes one virus species, *Kokobera virus*, with two strains: Kokobera virus (KOKV) and Stratford virus (STRV). These viruses have been isolated from mosquitoes in Australia and Papua New Guinea. Serological data suggest that kangaroos and horses may be reservoir hosts of KOKV. Human infections with KOKV occasionally result in an acute polyarthritic disease. STRV and two recently characterized novel viral species from Australia also provisionally assigned to this group do not seem to be pathogenic for vertebrates (Nisbet et al., 2005).

The Ntaya virus group currently comprises five viral species, *Bagaza virus* (BAGV), *Israel turkey meningoencephalitis virus*, *Ilheus virus*, *Ntaya virus* (NTAV) and *Tembusu virus* (TMUV). BAGV has been isolated from a number of mosquitoes predominantly in western and central Africa. Human or animal disease is unknown (Adam and Digoutte, 2005). Recently, one BAGV strain has been completely sequenced and previous assumptions that BAGV and ITV are synonymous viruses have been confirmed (Kuno and Chang, 2007). Israel turkey meningoencephalitis was first described in Israel in 1960 and the causative virus was identified shortly thereafter. Intriguingly, this disease has only been diagnosed in two countries so far, Israel and South Africa. In Israel, disease outbreaks correspond with the activity of mosquito vectors. ITV has also been isolated from unsorted pools of mosquitoes, however, the principal natural vector is not known. The disease occurs with highest incidence in turkeys 10–12 weeks of age. The affected birds show neurological signs and at pathological examination nonsuppurative meningoencephalitis and myocardial necrosis are found. In Israel, the disease is controlled by vaccination. ITV does not cause disease in humans (Guy and Malkinson, 2008). ILHV was one of the

earliest isolated flaviviruses. ICTV classification recognizes two subtypes, Ilheus virus and Rocio virus (ROCV). ILHV was recovered from mosquitoes at Ilheus city, on the eastern coast of Brazil. The virus is present in many regions of Central and South America. Birds are the only known hosts. ILHV is not associated with epidemic disease but it has been sporadically isolated from patients (from Brazil, Trinidad, Panama, Argentina, Colombia, Ecuador) with acute febrile illness with headache, myalgia and malaise. In three cases mild meningoencephalitis with photophobia was present (Karabatsos, 1985; Figueiredo, 2000; Shope, 2003). ROCV caused at least two human epidemics of severe encephalitis that lasted from 1973 to 1990 in the south-eastern region of Brazil. During these epidemics, more than 1000 clinical cases with approximately 100 deaths and more than 200 surviving patients with sequelae were recorded. There is serological evidence for continuing circulation of the virus in the region. Mosquitoes of the species *Psorophora ferox* and *Ochlerotatus scapularis* are laboratory-confirmed competent vectors. The natural reservoir hosts are not known. The factors leading to sudden emergence and disappearance of clinical cases remain enigmatic and there is concern that ROCV might reemerge one day (Figueiredo, 2000; Shope, 2003). NTAV has been isolated from several mosquito species in Africa (Uganda, Cameroon, Central African Republic). It has never been associated with diseases in humans or animals (Adam and Digoutte, 2005). TMUV has been recovered from mosquitoes (predominantly *Cx. tritaeniorhynchus*) in Malaysia and Thailand. The reservoir hosts are not known. Natural disease in humans and animals has not been recorded. However, an experimental infection of chickens with Sitiawan virus, a subtype of TMUV, resulted in encephalitis and growth retardation (Kono et al., 2000; Shope, 2003).

The Spondweni virus group includes only one virus species, *Zika virus*, with two subtypes, Spondweni virus (SPOV) and Zika virus (ZIKV). ZIKV is widely distributed in Africa and South-east Asia. It has been isolated from a number of mosquito species. There are isolates from monkeys, while birds do not seem to be involved in natural transmission of this virus (Adam and Digoutte, 2005). ZIKV has been repeatedly found to be responsible for human disease, partly as small outbreaks, the latest of which occurred in Yap state, Micronesia (Shope, 2003; Lanciotti et al., 2008; Hayes, 2009). Clinical signs include fever, headache, rash and arthralgia. Human cases of ZIKV infections might be underdiagnosed because clinical signs resemble other, more well known and widespread diseases such as dengue or chikungunya. Disease of animals is not known. SPOV seems to be present only in Africa, where it has been isolated from several mosquito species. There are single reports of human infections presenting with fever, headache, myalgia and rash. No animal reservoir host has been identified so far (Adam and Digoutte, 2005).

The last virus group belonging to the mosquito-borne flaviviruses is the Yellow fever virus group. This group includes the species *Banzi virus* (BANV), *Bouboui virus* (BOUV), *Edge Hill virus* (EHV), *Jugra virus* (JUGV), *Saboya virus* (SABV), *Sepik virus* (*SEPV*), *Uganda S virus* (*UGSV*), *Wesselsbron virus* and *Yellow fever virus*. BANV is one of the few mosquito-borne flaviviruses, the first isolation of which was from a human case, from the blood of a febrile child in South Africa. The virus has also been associated with another case of febrile illness in Tanzania. Neutralizing antibodies to BANV have been found in human sera from South Africa, Mozambique, Angola, Namibia and Botswana. The virus was also isolated from a few mosquito pools in different African countries, but also from cattle, rodents and hamsters in South Africa, Mozambique, Zimbabwe and Kenya. The natural hosts of BANV are probably rodents. BANV has not been associated with animal diseases (Fulop et al., 1995; Shope, 2003; Adam and Digoutte, 2005). BOUV was for the first time isolated from mosquitoes (*Anopheles paludis*) in the Central African Republic. Further successful isolations are from mosquitoes and several mammal species from different African states (Adam and Digoutte, 2005). There are no available informations on pathogenicity for man and animals. EHV was discovered in 1961 in *Oc. vigilax* mosquitoes near Cairns, Australia. The virus has since been recovered on multiple occasions from the same mosquito species and less frequently from other species. Wallabies have neutralizing antibodies to this virus which may point to their involvement in the natural transmission cycle of EHV. There is a single report on a probable human infection which was characterized by myalgia, arthritis and muscle fatigue. Disease in animals has never been reported (Shope, 2003). JUGV has been isolated from *Aedes* spp. mosquitoes in Malaysia in 1969. There is no information available on additional isolations and there are no sources providing information on transmission cycles or potential pathogenicity for warmblooded hosts (Calisher and Gould, 2003).

SABV is divided into two subtypes, Potiskum virus (POTV) and SABV. SABV has been isolated from a number of mosquito species, predominantly from Senegal and the Central African Republic. There are also several isolates from small mammals, but not from birds. Disease in animals as well as in humans is unknown (Adam and Digoutte, 2005). POTV was isolated in Nigeria from the liver of a giant rat (*Cricetomys gambianus*). After experimental infection, chicks developed a fatal disease. There are no reported isolations from mosquitoes and no informations concerning natural diseases in warmblooded hosts including humans (Omilabu et al., 1989).

SEPV has been isolated in 1966 from *Mansonia septempunctata* and later also from other mosquito species in New Guinea. There is one probable association with a human disease, which presented as fever and headache. A vertebrate host has not yet been identified and thus there is no indication for disease in animals (Shope, 2003).

UGSV has been isolated from a mixed pool of *Aedes* mosquitoes in Uganda. Several other isolates are from different mosquito species from West Africa, and there is one isolate from a bird and another isolate from the blood of a febrile patient (Tanzania), which are, however, not entirely confirmed (Adam and Digoutte, 2005). Generally, this virus appears not to be pathogenic to humans and other warmblooded hosts (Theiler and Downs, 1973).

In addition to JEV, WNV, USUV and ITV, WESSV is the fifth mosquito-borne flavivirus which causes animal

disease. As with JEV and ITV, high viraemia titers sufficient to infect mosquitoes are found in domestic animals (sheep, goats, cattle). WESSV was originally isolated in 1955 from an eight-day-old lamb during an episode of abortions and neonatal deaths in the Wesselsbron district, South Africa (Swanepoel and Coetzer, 2004). Shortly thereafter, the virus was also recovered from a human with a febrile disease and from a pool of *Ae. circumluteolus* in Tongaland, South Africa (Swanepoel and Coetzer, 2004). Presence of the virus has since been documented in vertebrates or mosquitoes from many African countries and also from Thailand (Shope, 2003; Swanepoel and Coetzer, 2004). The virus is pathogenic for new-born lambs, kids and pregnant ewes, and causes subclinical infections in non-pregnant adult sheep, goats, cattle, horses and pigs. In lambs and kids the disease is characterized by fever, anorexia and general weakness with a mortality rate of up to 27%. Pathological lesions include hepatic necroses and generalized haemorrhages. The infection of pregnant animals induced a febrile disease frequently followed by abortion. As WESSV activity is present in areas where also other viral diseases leading to comparable clinical problems are found (Rift valley fever, Nairobi sheep disease), Wesselsbron disease in animals is probably underreported. Human infections occur sporadically and are characterized by fever, headache, myalgia and arthralgia (Karabatsos, 1985). Most frequently humans seem to become infected by mosquitoes but laboratory infections and infections after handling carcasses of infected animals have also been described (Swanepoel and Coetzer, 2004).

YFV is estimated to be responsible for approximately 200,000 human clinical cases per year with a case fatality rate of approximately 20% (Barrett and Monath, 2003). The vast majority of cases occurs in Africa, but the virus is also active in South America. The virus is a native virus of Africa, which has most likely been transported to South America during slave trade in the early 1600s. It is maintained in an enzootic cycle involving monkeys and canopy-dwelling *Aedes* spp. in tropical rain forests, both in Africa and South America. Humans may be accidentally infected (“jungle yellow fever”). However, the virus is periodically introduced into urban areas where it is able to establish epidemics with *Ae.* (*St.*) *aegypti* as vector and humans as the only natural hosts. While many primate species involved in the transmission cycle seem to show subclinical infection, others, such as howler monkeys (*Alouatta* sp.) in Latin America or *Galago senegalensis* in Africa, may succumb to YFV infection. In primates (including humans) the virus is viscerotropic resulting in liver damage, but encephalitis is not a feature of this disease. A number of excellent reviews on different aspects of YF is available (Theiler and Downs, 1973; Gubler, 2002; Barrett and Monath, 2003; Gould and Solomon, 2008).

4. Conclusions and further prospects

Recent events, such as the introduction, rapid spread and permanent residence of WNV in the Americas, introduction and continuing transmission of a pathogenic strain of USUV in central Europe, introduction and establishment of a transient local transmission cycle of chikungunya virus (CHIKV) in Italy, or the emergence, spread and continuing transmission of Bluetonge virus serotype 8 in northwestern Europe, provide a number of examples for unexpected spread of certain mosquito-borne viruses to areas where they have never been recorded before and where efficient transmission cycles have been considered unlikely in the past. However, a number of factors in the globalized world, such as increasing and rapid transportation of people, animals, plants and other goods favour the worldwide spread of pathogens and their vectors. The first effects of global climate changes enable certain vector species to conquer regions previously unsuitable to them, e.g. *Ae. albopictus* (*St. albopicta*), a competent vector for DENVs and CHIKV, has been proven to be present in rather temperate areas of Italy and is on the move to the north. On the other hand, mosquito species considered to be vectors with low competence, can play increasing roles in transmission cycles because rising average temperatures also increase the replication rate of pathogens in certain vectors (Purse et al., 2005).

For many of the viruses mentioned above very little information is available. This was also the case for USUV until 2001, when it appeared for the first time and totally surprisingly in Europe. With that event we may have been lucky, because that particular virus strain showed no considerable pathogenicity for humans. However, a number of mosquito-borne viruses is around in different habitats almost worldwide and it is most likely only a matter of time till a more pathogenic phenotype of one of these viruses spreads to new habitats, where it will again meet unprepared and immunologically naïve host populations. Currently, from a large number of hitherto considered unimportant viruses, only partial sequences of the genomes are available and in most cases only from one strain. Taking into account the strain diversities of the better characterized viruses, such as JEV, WNV, DENV, or YFV, it is very likely that also the poorly characterized viruses show a comparative level of diversity, including mutations which might give rise to more pathogenic phenotypes. Thus it is a matter of high priority to generate sequences of entire genomes, not only of reference strains (frequently isolated decades ago) of these viruses but also of more recent isolates.

Conflict of interest statement

None.

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