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OPINION OF THE SCIENTIFIC COMMITTEE ON MEDICINAL PRODUCTS AND MEDICAL DEVICES ON

"THE IMPACT OF ARTHROPOD BORNE DISEASES (INCLUDING WNV) ON THE SAFETY OF BLOOD USED FOR TRANSFUSION AS WELL AS ORGANS USED FOR TRANSPLANTATION IN THE EUROPEAN COMMUNITY"

Adopted by the SCMPMD during the 24th plenary meeting of 16 October 2003

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1. Background

There are many different members in the group of arthropod-borne viruses (arboviruses). Common to all arboviruses is their transmission by arthropods, e.g. mosquitoes or ticks, to vertebrates, e.g. rodents or birds. In most instances, the virus replicates optimally in a single or in a few closely related species of insects which constitute the major vector. The mammalian or avian species involved in the viral maintenance cycle are usually limited because most blood-feeding insects have a clear feeding preference for only few vertebrate species.

Humans comprise the major vertebrate host for only very few arboviruses (e.g. Dengue virus, Urban Yellow Fever virus). However, there are also instances where humans are accidentally infected, e.g. by St Louis Encephalitis Virus, Tick Borne Encephalitis Virus or by West Nile Virus, when the tick or mosquito happens to feed on humans instead of the preferred vertebrate host (rodents or birds).

West Nile virus (WNV) has been recognised since 1937 when it was first isolated in Uganda and the virus is widely distributed throughout Africa, Western Asia, Europe and Australia. Less than 1% of infected humans develop WNV associated diseases, with fatal encephalitis or meningitis as the worst cases. Recent attention has been drawn to WNV in the USA and Canada, where this virus has caused yearly epidemics throughout the summer months since 1999. The time course of these epidemics parallels the life-cycle of the mosquitoes, and there has been a regular increase in the outbreak from 1999 to 2002.

Preliminary data available for the USA in 2003 indicates an increased epidemic course compared with the year 2002 when 4,156 human cases and 284 deaths on WNV-caused encephalitis were observed. Cases of secondary WNV transmissions by blood transfusion and organ transplantation have been reported.

Within Europe, outbreaks have been reported in Russia, Romania, Italy (in 1998) and the South of France (2000). However, the reported cases in Italy and France involved only horses and no human cases were observed.

Currently only very limited data are available for most EU countries with regard to the prevalence of human WNV infections or the incidence of new infections.

2. Mandate

The SCIENTIFIC COMMITTEE ON MEDICINAL PRODUCTS AND MEDICAL DEVICES was asked to give an opinion concerning the potential impact of arthropod borne diseases (including WNV) on the safety of blood for transfusion and organs used for transplantation in the European Community.

In parallel, The Scientific Committee on Veterinary Measures relating to Public Health was asked to review the present knowledge on the WNV epidemiological situation in Europe. Their Opinion has been adopted on April 14-15, 2003. In order to avoid unnecessary overlap of the two Opinions, reference is made to information already contained in that Opinion, especially in respect to WNV epidemiology, WNV diagnostic tools and WNV sentinel systems.

3. Introduction

Arthropod-borne viruses are characterised by continuous cycling of the virus between insect and vertebrate (mammalian or avian) hosts. Viral replication occurs in both the insect and the vertebrate host, and both hosts are necessary for the maintenance of the viral life cycle. Furthermore, so-called "dead-end" or terminal hosts are known which may develop arbovirus-associated disease but are not involved in maintaining the virus cycle. Currently, more than 400 different arboviruses are known, all of which are lipid enveloped and belong to one of four virus families:

(1) *Arenaviridae* (e.g. Lymphocytic Choriomeningitis Virus),

(2) *Bunyaviridae* (e.g. Bunyavirus Bwamba virus, the Nairoviruses Crimean-Congo haemorrhagic fever virus, Nairobi sheep disease virus, Dugbe virus, Phlebovirus Rift Valley fever virus, Toscana virus)

(3) *Flaviviridae* (e.g. Yellow Fever Virus, West Nile Virus, Tick Borne Encephalitis Virus)

(4) *Togaviridae* (e.g. the Alphaviruses causing Eastern and Western equine encephalitis, Ross River virus, Ockelbo virus).

4. Flaviviruses

The flavivirus family comprises the most prominent group of arboviruses which are potentially pathogenic for humans. This Opinion will therefore focus on flaviviruses though some members of the other arbovirus groups are also pathogenic for humans, too. Annex 1 provides list of currently known flaviviruses and their vector and host systems.

Members of the flavivirus family share physico-chemical features such as the virus particle size of approximately 50 nm, the presence of a lipid envelope and the organization of the viral genome. The single-stranded (+) RNA genome encodes a polyprotein which is cleaved into non-structural (enzymatic) and structural moieties.

4.1. Dengue virus

Different flaviviruses may infect humans as dead-end vectors and cause different diseases, e.g. fever, arthralgia, rash and haemorrhagic fever. Humans are one of the natural hosts for only a few members of this virus family. An example is Dengue Virus which is transmitted by mosquitoes (*Aedes aegyptii*) and causes in humans Dengue fever (DF) or, upon re-infection with a different serotype, the clinically more severe Dengue hemorrhagic fever or Dengue shock syndrome. Four different Dengue virus serotypes with no immunological cross protection have been described and they have caused numerous infections in Asia during World War II. With the constant increase of aeroplane travel during the 2nd half of the last century Dengue virus infected mosquitoes were transported to South and North America and have caused several outbreaks. With 50 – 100 million new cases / year world-wide [(1)] DF is now considered the virus infection most frequently transmitted to humans by insects. The pathogen is endemic in most of the tropical cities, many of which are popular tourist destinations. On a worldwide scale the distribution of DF cases correlates well with the distribution of the specific vector, *Aedes aegyptii*.

As with other flaviviruses which may infect humans there is a short viremic phase prior to the onset of symptoms. Therefore, the virus might be transmitted by blood transfusion from a recently infected donor to recipients of respective blood components (erythrocytes, thrombocytes, non-virus inactivated plasma). A recent instance of Dengue virus transmissions by a blood donation has been documented by the blood transfusion service in Hong Kong [2]. Two recipients were infected through blood components originating from an apparently healthy blood donor who had been infected few days before donating blood. Although travellers repeatedly import Dengue virus into Europe [3], in the absence of the insect vector, the infection did not become endemic. There has been no documented transmission of DF by blood donations in Europe as yet. However, the incidence of imported DF in Europe is certainly underestimated because of lack of surveillance and lack of standardized diagnostic systems

4.2. Tick borne encephalitis virus

Tick borne encephalitis (TBE) viruses are flaviviruses with different small rodents as natural reservoir, different ticks as transmitting insects and humans as dead-end hosts. TBE occurs in rare cases as a fatal disease in an endemic pattern over a wide area of Europe and the former Soviet Union. The distribution corresponds to the distribution of the respective tick species, *Ixodes ricinus* (vector of Central European encephalitis virus) and *Ixodes persulcatus* (vector of Russian spring-summer encephalitis). These two TBE viruses are closely related antigenically and have been considered subtypes of the same virus. Transmission to humans has been described not only through tick bites but also by consumption of unpasteurized cow, goat or sheep milk or cheese [4, 5]. Goats, sheep and cattle are hosts for adult *Ixodes* ticks, have low level viremia and are therefore not considered to play a role in TBE virus cycle. Different kinds of TBE vaccines, some of them associated with side-effects, have been available since 1976. In Austria, mass vaccination was initiated in 1981 resulting in a significant decline of the annual incidence of disease; in other EU Member States TBE vaccines are recommended mainly for persons living in endemic regions, working under high-risk conditions (foresters, woodcutters, laboratory workers) or travellers visiting high-risk areas.

Prior to the first signs of a TBE virus infection (flu-like symptoms, fever) a low level viremia is present, as it was demonstrated e.g. in experimentally infected animals [6]. There are no reports of TBE cases originating from blood transfusions in spite of the following elements: a relatively high TBE prevalence in the past in some European countries, e.g. Austria, the detection of the virus in the blood during the initial asymptomatic course of infection and reports about food-associated TBE infections. Similar to the situation for Dengue viruses, this might be explained by a lack of standardized diagnostic procedures, by mild courses of disease and by suboptimal effectiveness of surveillance systems for recipients of blood transfusions.

4.3. West Nile Virus

West Nile virus (WNV) is another flavivirus with a natural life cycle involving many species of birds and ornithophilic mosquitoes, particularly, but not exclusively, *Culex* species. WNV susceptible avian species include birds which migrate between Africa, Europe and Asia and non-migratory birds, all of which can become infected when WNV-infected mosquitoes feed on them. Humans, horses and other mammals may become infected by WNV as dead-end hosts, with WNV transmitted by *Culex* mosquito bites.

In humans, WNV infection is either non-symptomatic (80%) or is followed by mild symptoms, such as fever or headache. In few cases WNV-infection may also be associated with encephalitis with a potentially fatal outcome in primarily elderly patients. The overall frequency of fatal outcomes is less than 1% of observed infections.

5. WNV Epidemiology

WNV was isolated for the first time in 1937 from the blood of a febrile woman in the north of the Uganda, in a region close to a tributary of the river Nile [7]. Today WNV is known to be widely distributed throughout Europe, Africa, Asia and, since 1999, also in North America.

In the early 1950s WNV was isolated from patients, birds and mosquitoes in Egypt [8]. Recognised since the 1960s in Europe, this agent became very well known to the public at large in 1999, after its first isolation on the American continent. The American outbreak is characterised by a high mortality rate among birds and the virus has spread from New York to all North American states in three years during the season from May to September, due to mosquito activity.

5.1. Europe

The first indication for WN virus in Europe was reported in 1958 from Albania (two out of 112 healthy Albanians had neutralizing antibodies) [9] . Following this report many other European countries, mainly in the Mediterranean area, also found proof of WNV presence, namely: Bulgaria, Czech Republic, France, Moldavia, Portugal, Romania, Russia, Slovakia and Ukraine. In Europe, virus was isolated in 1963 from patients and mosquitoes in France (Rhône Delta) [10], in 1964 from patients and ticks in The Volga Delta [11], in 1969 from mosquitoes in Portugal [12], and subsequently in Slovakia, Moldavia, Ukraine, Hungary, Romania, Czech Republic and Italy. There have been several reports of self-limiting WNV endemics in these countries over the last decades. The different surveillance systems established in some European countries include serosurveillance of different vertebrate species and investigations on clinical cases (horses, humans). A summary of past WNV activity in Europe and Israel is provided in Annex 2.

Vectors

In Europe, the principle cycle of WNV circulation is a rural, sylvatic cycle with transmission between wild, usually wetland, birds and ornithophilic mosquitoes. A second urban cycle may also occur (as in the outbreak in Bucharest, 1996-97), between urban birds and mosquitoes (mainly *Culex pipiens/molestus).* A bird-tick (*Argasidae* or *Ixodidae family)* cycle may occur in some dry warm habitats which does not contain mosquitoes. In Europe WNV was isolated from 8 species of mosquitoes (*Culex pipiens, C. modestus, Mansonia richiardii, Aedes cantans, A. caspius, A. excrucians, A. vexans and Anopheles maculipennis*) and two species of herd ticks (*Hyalomma marginatum and Dermacentor marginatus*) [13] .

Hosts

The virus was isolated from 11 wild aquatic and terrestrial avian species (*Ardeola ralloides, Plegadis falcinellus, Anas querquedula, Fulica atra, Tringa ochropus, Vanellus vanellus, Larus ridibundis, Streptopelia turtur, Corvus frugilegus and Sturnus vulgaris,* all migratory and *Corvus corone* non-migratory).

A very recent publication [14] summarizes data obtained in the United Kingdom with the serological analysis of 353 bird sera collected from 30 different wild or farm birds.

The analysis of neutralizing antiviral antibodies revealed that in total 52 / 353 birds (14.7%) had an exposure history to WNV or to Usutu Virus which is another flavivirus closely related to WNV. The bird species testing anti-WNV-positive included both migrant and resident bird populations, implying

efficient WNV transmission not only in warmer countries but also in the UK. There was no obvious reduction in the overall bird population by WNV-associated diseases observed which is in contrast e.g. to the USA experience. Possible explanations are either that avirulent WNV strains have been introduced into the UK or that local birds have been exposed to WNV for some time and have developed herd immunity. The second explanation seems to be more plausible since sequence analysis of WNV genomes detectable in some bird brains revealed no major differences when compared with US data. North American avian (and horse) species seem to develop frequently fatal infections to WNV, presumably because it was introduced to the United States for the first time in, or just before, 1999 and no pre-existing herd immunity was present.

5.2. USA

On the 6th October 1999 an outbreak in New York City reported previously as being caused by St. Louis encephalitis Virus was confirmed to be due to WNF, according to the WHO Communicable Disease Surveillance and Response (CSR). To that date 50 previously reported St. Louis encephalitis cases had been confirmed as WNF with 5 deaths among older adults [15].

The seasonal endemics increased from year to year, and WNV surveillance provided evidence for an East-West migration affecting different states and Canada. In 2002, the last update of the CDC [16] reports 4.156 human cases of WN virus infection, with 284 fatalities. During 2002, WNV activity (evidence of infections in birds, humans, mosquitoes, and other animals – primarily horses) has been documented in 43 states and the District of Columbia.

In 2003, the currently latest update of CDC (October 10, 2003; with 6,507 human cases including 136 fatalities) [17] shows that during this year the situation in regard to WNV seems to be similar or even worse compared to the year 2002. The epidemic moved further West and many human WNV infections occurred in States which had only very few WNV cases in 2002 (e.g. Colorado with 14 cases in 2002 [16] and currently 2,090 cases in 2003 [17]). According to CDC, 65 per cent of cases occur from mid-August to late September because that is also the high activity season for the mosquito population and because many people are outdoors at that time. A comparison with last year's numbers is difficult. Now that additional data from WNV screening of blood donors becomes available, and the general awareness of WNV as a new pathogen has increased, these factors have certainly resulted in some reporting bias.

6. WNV transmission by blood transfusion and organ transplantation

During the typical course of mosquito-borne West Nile virus infection a viremic phase (in many cases asymptomatic) of 2 – 14 days precedes the WNV specific humoral response and subsequent virus clearance from blood [18, 19]. This phase may be prolonged (7 – 31 days) in immunocompromised patients infected with WNV by transfusion of erythrocytes, thrombocytes or fresh frozen plasma [20]. Blood components collected from a donor who is in the viremic phase at the time of donation are believed to be potentially infectious to the transfusion recipient unless the blood products undergo virus inactivation procedures. New virus inactivation procedures for cellular components (erythrocytes, thrombocytes) or fresh frozen plasma undergoing quarantine storage have still not been introduced on a large scale, into Europe.

In contrast to cellular components and non-virus inactivated therapeutic plasma, pooled plasma used for the fractionation of plasma derivatives, e.g. immunoglobulins, albumin or coagulation factor concentrates, undergoes virus inactivation and/or removal procedures. These reduction procedures as applied during manufacturing of plasma derivatives have been validated for their efficiency against non-enveloped and enveloped viruses. The efficiency of these measures has been proven convincingly for hepatitis C Virus (HCV) which is another member of the Flaviviridae. Before the EU-wide introduction of the respective nucleic acid amplification techniques (NAT) for the testing of plasma pools for HCV-genomes in 1999 [21] HCV was frequently present in plasma pools from different manufacturers [22].The viral burden of this source material originated from single highly viremic (up to 10^8 copies HCV-RNA / ml plasma) units collected during the diagnostic window phase. Nevertheless, no HCV transmissions were observed with plasma derivatives which were manufactured with the inclusion of potent virus inactivation and/or removal steps. For WNV, the donations with highest viremia detected so far harboured virus concentrations in the range of 10^5 copies WNV-RNA / ml viremic plasma. Also, additional studies investigating specifically WNV inactivation have confirmed the expected efficiency of virus inactivation [23].There is thus no risk of WNV transmission through virus-inactivated plasma products. This conclusion was recently confirmed by the CPMP (EMEA) as a Position Statement on West Nile Virus and Plasma derived medicinal products [24].

Estimations of the risk for WNV transmission through blood transfusion during an epidemic in the US revealed expected frequencies of up to 2.7 transmissions / 10,000 blood transfusions, with a seasonal peak in late August [25]. In 2002 several cases of WNV transmissions by blood transfusion in the US were reported by surveillance systems [20, 26]. Some of these had fatal outcomes, especially in elderly or immunocompromised recipients. Only a minority of the involved donors gave a (retrospective) history of (mild) symptoms consistent with WNV infection. New follow-up data from 510 viremic donors show that five of them subsequently had meningoencephalitis and 72 West Nile fever [17].

Another potential way for secondary WNV infections are organ transplantations: WNV transmissions were reported for all four recipients of organs originating from one donor [27]. The organ donor again had received prior to donation blood transfusions from 63 donors. On retrospective testing one blood donor was found to be WNV-RNA positive at the time of the donation, followed by appearance of antibodies. This study confirms the high WNV infection-associated transfusion risk and a more severe disease outcome in recipients who are on immunosuppressive drugs.

7. WNV specific measures in the USA

In 2002 FDA recommended to the diagnostics industry the accelerated development of *in vitro* diagnostics suitable for WNV screening of the American blood supply. Since the viremic phase precedes the anti-WNV (IgM/IgG)-positive period, nucleic acid amplification techniques (NAT) for the detection of the viral genomic RNA were considered as the most promising tool for the identification of infectious blood donations. Until the (expected) beginning of this year's WNV season three different NAT systems had been developed and were accepted by FDA to be used for blood screening from 1 July 2003 onwards. All three systems have detection sensitivities in the range of 6 – 10 copies WNV-RNA/ml plasma, are designed for use with pooled plasma specimens and, nevertheless, are reasonably well capable of detecting low-titre viremic units.

Two of the NAT systems are based on technologies which are already used in American blood collection centres for the NAT screening of HCV and HIV. These are the transcription mediated amplification method which has been developed by GenProbe for the isothermal amplification of target nucleic acids, and the TaqMan PCR system of Roche Molecular Systems. The third method is a PCR system provided at the facilities of National Genetic Institute.

Preliminary results demonstrate the efficiency of these NAT screening systems: of the approximately one million donations screened to 5th Aug 2003, a total of 163 (about 0.015 per cent of total donations) were repeatedly NAT reactive [28]. Results of additional NATs for a further 28 screening test-reactive donations were pending at that time. These numbers from "off-peak" donations are even higher than calculated by theoretical estimations [25] and could reflect an increase of the WNV epidemic in the USA in 2003 when compared to 2002. More recent data give a total number of 654 NAT-reactive donors by October 8, 2003 [17].

8. Conclusions

There are a variety of different members in the group of arthropod-borne group of viruses. Some of them, such as TBE virus, are endemic in parts of Europe, others, such as Dengue, have been imported occasionally without causing major endemics. WNV has been present in different Member States and historically has caused self-limiting local endemics in different host species. Although consistent surveillance data are not available across all of Europe, there is convincing evidence that WNV was present in the past in Europe on several occasions and is still present at least in some host species, without causing severe disease. At present it is uncertain if this can be explained by herd immunity of hosts (e.g. birds in the UK) which have been exposed repeatedly to avirulent WNV strains or by climatic conditions which are suboptimal for an efficient transmission of the virus.

There is serious concern, in EU Member States, that WNV might be introduced into the blood donor pool in European countries, possibly by individuals with a recent travel history to WNV endemic areas. Although comparative incidence data for the European blood donor population regarding both WNV and endemic arboviruses (e.g. TBE viruses) are missing, this concern grows in parallel with the annual increase of the WNV epidemic in the USA and in Canada.

Similar concern applies to the potential transmission through transplants. There is clear evidence from recent publications that the severity of WNV disease is increased in the immunosuppressed recipients of transplants. However, because of very limited availability of organs for transplantation and the corresponding urgent need, the risk of WNV transmission should be weighed against the benefits of organ transplantation.

There are a range of approaches to protect the public from transfusion- and/or transplant-associated infections. Some of them are specific for individual pathogens, others comprise a more general risk reduction.

The possible approaches include the exclusion of individuals who are estimated to be at higher risk for a recent infection from blood or transplant donation. This risk could be defined by asking potential blood donors for the mild symptoms consistent with a recent WNV infection and/or by asking for recent travel history in endemic regions.

Another approach is the introduction of procedures for the inactivation of pathogens potentially present in blood donations. This approach is currently handicapped by a lack of long-term

experience with newly available inactivation systems and by an associated significant increase of costs.

The screening of blood for the specific marker or, if possible, for a non-specific marker for recent infections is another option to reduce the risk of transfusion- or transplant associated infections. Again, costs for NAT screening systems are very high, and a potentially suitable non-specific marker for the majority of WNV infections has not yet been identified.

Decision making to determine which is the most appropriate approach is based on a range of considerations and factors such as: the ease of introduction, the scarcity of organ transplants; potential ways to cope with less blood donations; the urgency of organ transplants and blood components; the epidemiology of the pathogen combined with an assessment of the likelihood of its presence in the blood donor population; the probability for development of severe disease upon infection; the availability of new technologies; cost-benefit analysis for the introduction of new test and/or inactivation systems.

9. Recommendations

a) Since the WNV epidemic in the USA (and Canada) reappeared at levels similar to the 2002 epidemic also in 2003, the temporary exclusion of travellers from WNV affected regions from blood donation is considered a well-balanced measure, with a four weeks interval between the last day spent in an endemic region and the possible date of a blood donation being considered sufficient.

b) Further pathogens with a short asymptomatic viremic carrier state may be introduced into Europe from other parts of the world by travellers (e.g. Dengue virus). Therefore, a four weeks deferral of travellers returning from outside Europe from blood donation might be considered. This general rule would avoid future repetition of discussions and decision making process when outbreaks of infection cause endemic infection in non European Countries.

c) The data regarding the current epidemiology of WNV and other arboviruses in Europe are inadequate. Furthermore, available data are difficult to compare because of inconsistencies in their respective approaches. The decision making regarding the introduction of measures appropriate for reducing the risk of WNV or other arbovirus infections by blood transfusion and/or organ transplantation is thus difficult. Therefore high priority should be put into research programs on prevalence and incidence of arbovirus infections in the EU for the assessment of relative risks on a standardised data basis.

10. Annex 1: The Flaviviruses: vector-host relationships and disease associations

(from B.N. Fields, Virology, 3d edition, Lippincott and Raven (Philadelphia, New York), pp.962-963, modified)

Virus	Vector[a]	Principal vertebrate host	Geographic distribution	Human disease	Animal disease
Absettarov[b]	Tick	Rodent	Eur.	+	
Alfuy	Mosquito	Bird	Austr.		
Apoi		Rodent	Asia	+[c]	
Aroa	?Mosquito		SA		
Bagaza	Mosquito		Afr.		
Banzi	Mosquito		Afr.	+	
Bouboui	Mosquito	?Monkey	Afr.		
Bussuquara	Mosquito	Rodent	SA	+	
Cacipacore		Bird	SA	+	
Carey Island		Bat	Asia		
Dakar bat		Bat	Afr.	+	
Dengue 1	Mosquito	Human, monkey	WW	+	
Dengue 2	Mosquito	Human, monkey	WW	+	
Dengue 3	Mosquito	Human	WW	+	
Dengue 4	Mosquito	Human	WW	+	
Edge Hill	Mosquito	?Marsupial	Austr.	+	
Entebbe bat		Bat	Afr.		
Gadgets Gully	Tick	?Bird	Austr.		
Hanzalova[b]	Tick	Rodent	Eur.	+	
Hypr[b]	Tick	Rodent	Eur.	+	
Ilheus	Mosquito	Bird	SA	+	
Israel turkey meningo-enc.	Mosquito	Bird	ME, Afr.		Turkey
Japanese encephalitis	Mosquito	Bird, pig	Asia	+	Pig, horse
Jugra	Mosquito	Bat	Asia		
Jutiapa		Rodent	CA		
Kadam	Tick	?Rodent	Afr., ME		
Karshi	Tick	?Rodent	Asia		
Kedougou	Mosquito		Afr.	+	
Kokobera	Mosquito	?Bird	Austr.	+	
Koutango	Tick, (?Mosquito)	Rodent	Afr.	+	
Kumlinge[b]	Tick	Rodent	Eur.	+	
Kunjin	Mosquito	Bird	Austr.	+	Horse
Kyasanur Forest disease	Tick	Rodent	Asia	+	
Langat	Tick	?Rodent	Asia	+[d]	

Virus	Vector[a]	Principal vertebrate host	Geographic distribution	Human disease	Animal disease
Louping ill	Tick	Bird, rodent	Eur.	+	Sheep, pig, horse, goat, deer, dog, grouse
Meaban	Tick	Bird	Eur.		
Modoc		Rodent	NA	+	
Montana myotis leuko-encephalitis					
Murray valley encephalitis	Mosquito	Bird	Austr.	+	?Horse
Naranjal	Mosquito	?Rodent	SA		
Negishi	Tick		Asia	+	
Ntaya	Mosquito		Afr.		
Omsk hemor-rhagic fever	Tick	Rodent	Asia	+	Muskrat
Phnom-Penh bat		Bat	Asia		
Powassan	Tick (Mosquito)	Rodent	NA, Asia	+	
Rio Bravo		Bat	NA	+	
Rocio	Mosquito	Bird	SA	+	
Royal Farm	Tick	Bird	Asia		
Russian spring-summer enc.	Tick	Rodent, bird	Asia, Eur.	+	
Saboya	?Phlemoto-mine	Rodent	Afr.		
St. Louis encephalitis	Mosquito (Tick)	Bird	NA, CA, SA	+	
Sal Vieja		Rodent	NA		
San Perlita		Rodent	NA		
Suamarez Reef	Tick	Bird	Austr.		
Sepik	Mosquito		Afr.	+	
Sokuluk		Bat	Asia		
Spondweni	Mosquito		Afr.	+	
Stradford	Mosquito		Austr.		
Tembusu	Mosquito	?Bird	Asia, Austr.		
Tyuleniy	Tick	Bird	Asia, NA		
Uganga S	Mosquito	Bird	Afr.		
Usutu	Mosquito	Bird	Afr.	+	
Wesselsbron	Mosquito (Tick)	?Rodent, sheep	Afr., Asia	+	Sheep
West Nile	Mosquito (Tick)	Bird	Afr., Eur., Asia	+	Horse
Yaounde	Mosquito	Rodent, bird	Afr.		

Virus	Vector[a]	Principal vertebrate host	Geographic distribution	Human disease	Animal disease
Yellow fever	Mosquito (Tick)	Monkey	Afr., SA	+	
Zika	Mosquito	Monkey	Afr., Asia	+	

[a] isolation from alternate vector, but uncertain role in natural transmission cycle.
[b] closely related or identical to Russian spring-summer encephalitis.
[c] Laboratory infection only
[d] Disease following experimental infection for cancer therapy.

Afr., Africa; Austr., Australia-New Guinea; CA, Central America; Eur., Europe; ME, Middle East; NA, North America; SA, South America; WW, world-wide

11. Annex 2

Epidemiological Surveys for West Nile Virus in Europe and Israel

Country	Seroepidemiological surveys			Clinical cases / outbreaks
	Birds	**Horses**	**Humans**	
Portugal	**1969-70:** 3.25% of 400 bird sera (39 species) antiWNV-pos, including non-migratory species [31]	**1962-65:** 7 out of 24 horses (29%) with history of encephalomyelitis were antiWNV-pos [32] **(Cattle 1966-67:** 16% of 1294 bovine sera antiWNV-pos) [29, 30]	**1973:** 8 / 1649 sera (0.5%) with neutralizing antiWNV [33]	
Spain			**1973**: 17% of 701 sera antiFlaviv.-pos [34] **1975-76:** 8% of 1037 sera antiWNV-pos [35] **1980**: 8 % of 130 sera from Ebro delta region antiFlaviv.-pos [36] **2002:** 1 % of 797 sera from Ebro delta region antiFlaviv.-	

Country	Seroepidemiological surveys			Clinical cases / outbreaks
	Birds	**Horses**	**Humans**	
			pos	
France		**1963-64:** 6 / 37 horses (16%) without clinical symptoms and 6 / 10 horses with previous disease with neutralizing antiWNV-Abs [39] **1975-79:** 2 / 99 (2%) antiWNV-pos [37] **2000:** 8.3% of 5.133 horse sera antiWNV IgG-pos, many with IgM, too [38]	**1975-79** 4.9% of 235 sera antiWNV-pos [37]	**Horses** **1962-65:** 50 clinical cases in Camargue region (Bouches-du-Rhône) [39] **2000:** 76 clinical cases in "Little Camargue" (Herault Province) [40] **Humans** **1962-64:** 13 clinical cases, some with encephalitis, in Camargue region [41] **1982:** Imported case with encephalitis and severe myelitis from Israel [42]
Italy				**Horses** **1998:** 14 clinical cases in Tuscany [43]
Romania	**1966:** 30 / 73 (41%) of domestic fowl and 1 / 12 Passeriforms antiWNV-pos [45]		**1998:** 4% of 959 Bucharest residents antiWNV IgG-pos [44]	**Humans** **1996-97:** 352 individuals with acute aseptic meningitis and encephalitis caused by WNV outbreak near Bucharest [46]

Country	Seroepidemiological surveys			Clinical cases / outbreaks
	Birds	**Horses**	**Humans**	
				1997-98: 12 clinical cases in South-Eastern Romania with meningoencephalitis, 1 fatal [47]
Czech Republic				**Humans** **1997:** 5 clinical cases **1999:** 13 / 619 (2%) individuals with neutralizing antibodies, 4 clinical confirmed cases; associated with heavy rains and increases in *Aedes* mosquito population [48]
Russia and Ukraine				**Humans** **1980:** Ukraine: clinical cases, antibodies detected in 4,3% of fever patients [49] **1999:** In Volgograd region 183 cases (480 estimated) with acute aseptic meningoencephalitis, meningitis or acute fever [50]
Israel		**1959-60:** 28 / 81 (35%) antiWNV-pos [51] **1998:** 18 / 24 (75%) antiWNV-pos [52]		**Humans** **1951-52:** first clinical cases described with fever, malaise and general weakness, rarely signs of mild meningeal involvement [53, 54] **1953:** Clinical illness with fever together with enlarged

Country	Seroepidemiological surveys			Clinical cases / outbreaks
	Birds	**Horses**	**Humans**	
				lymph nodes and/or rash [55] **1957:** Clinical illness [56] **1959:** Clinical illness of laboratory worker by aerosol [57] **1962:** Four clinical cases with encephalitis [58] **1969:** One clinical case with fever, rash, lymphadenopathy, severe abdominal pain, leucopenia and pancreatitis [59] **1977:** Clinical case with fever, enlarged lymph nodes and acute myelitis [60] **1980:** Three clinical cases with fever and signs of meningeal irritation [62] **1999**: Two cases with encephalitis, one fatal [61] **2000:** Ministry of Health in Israel has reported 151 cases of WN fever with 76 cases hospitalized and 12 deaths (as of 19.09.2000) [63] **2000:** Outbreak with 417 antiWNV-pos cases and 33 deaths [64]

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