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OPINION OF THE

SCIENTIFIC COMMITTEE ON VETERINARY MEASURES RELATING TO PUBLIC HEALTH

ON

WEST NILE VIRUS (WNV)

(adopted on 14-15 April 2003)

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1. BACKGROUND

West Nile virus (WNV) is an arthropod-borne virus (*Flavivirus*) first isolated in Uganda in 1937 and widely distributed throughout Africa, Western Asia, Europe, Australia and the Middle East and recently reported in the USA and Canada.

WNV has been detected in the USA since 1999 and the incidence of human West Nile virus infection is rapidly increasing- cases have already occurred in 38 US States.

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

The virus is normally transmitted by mosquitoes, which suck the blood of infected birds and then feed on humans or other animals such as horses.

Recently there have been unpublished reports that WNV antibodies have been detected in non-migratory birds in the UK, indicating the presence of the virus in that population. However to date, there have been no cases of the disease reported in the country.

2. MANDATE

The Scientific Committee on Veterinary Measures relating to Public Health is asked to review the present knowledge on the epidemiological situation in Europe with regard to West Nile Virus, including whether *Equidae*, and / or other animals can be used as a sentinel or indicator for the Public Health risk.

3. INTRODUCTION

West Nile virus (WNV) is a mosquito-borne flavivirus transmitted in natural cycles between birds and mosquitoes, particularly *Culex* species mosquitoes.

In humans, WNV infection is a non-symptomatic or mild febrile illness; however encephalitis cases are reported with some fatalities particularly in elderly patients. WNV is also a cause of animal disease, especially in horses and birds.

WNV was first discovered in 1937 in the blood of a native woman of the West Nile District of Uganda who was suffering from a mild febrile illness. Since then, both sporadic cases and major outbreaks of WNV fever have been reported in Africa, the Middle East, Europe and Asia and many cases of WNV infection have been well documented since the early 1950s in Egypt and Israel, in the 1960s in France, and in the 1970s in South Africa. However during the last five years reports of WNV virus have been published, due to outbreaks occurring in Romania, Morocco, Italy, Russia and Israel but more especially, with the discovery of the virus in North-America in 1999.

4. EPIDEMIOLOGY

4.1. WNV and strains

Based on nucleic acid sequences using primers located in the envelope gene fragment, phylogenetic trees grouped most of the isolates into two major lineages that diverged by up to about 30% in nucleotide sequence (Berthet *et al*., 1997). European isolates clustered into lineage I whereas African isolates clustered into lineage I and II (Figure 1).

The addition of isolates from the recent outbreaks has confirmed this clustering into two lineages: isolates from Africa are present in both lineages, whereas isolates from Europe, Mediterranean Basin and India are exclusively in lineage I and isolates from Madagascar are in lineage II). This indicates that European epidemics may be initiated by introduction of variants from Africa through migratory birds, although introduction from Europe to Africa cannot be ruled out. The reasons for the absence of lineage II isolates outside Africa and Madagascar remain unknown.

4.2. Hosts

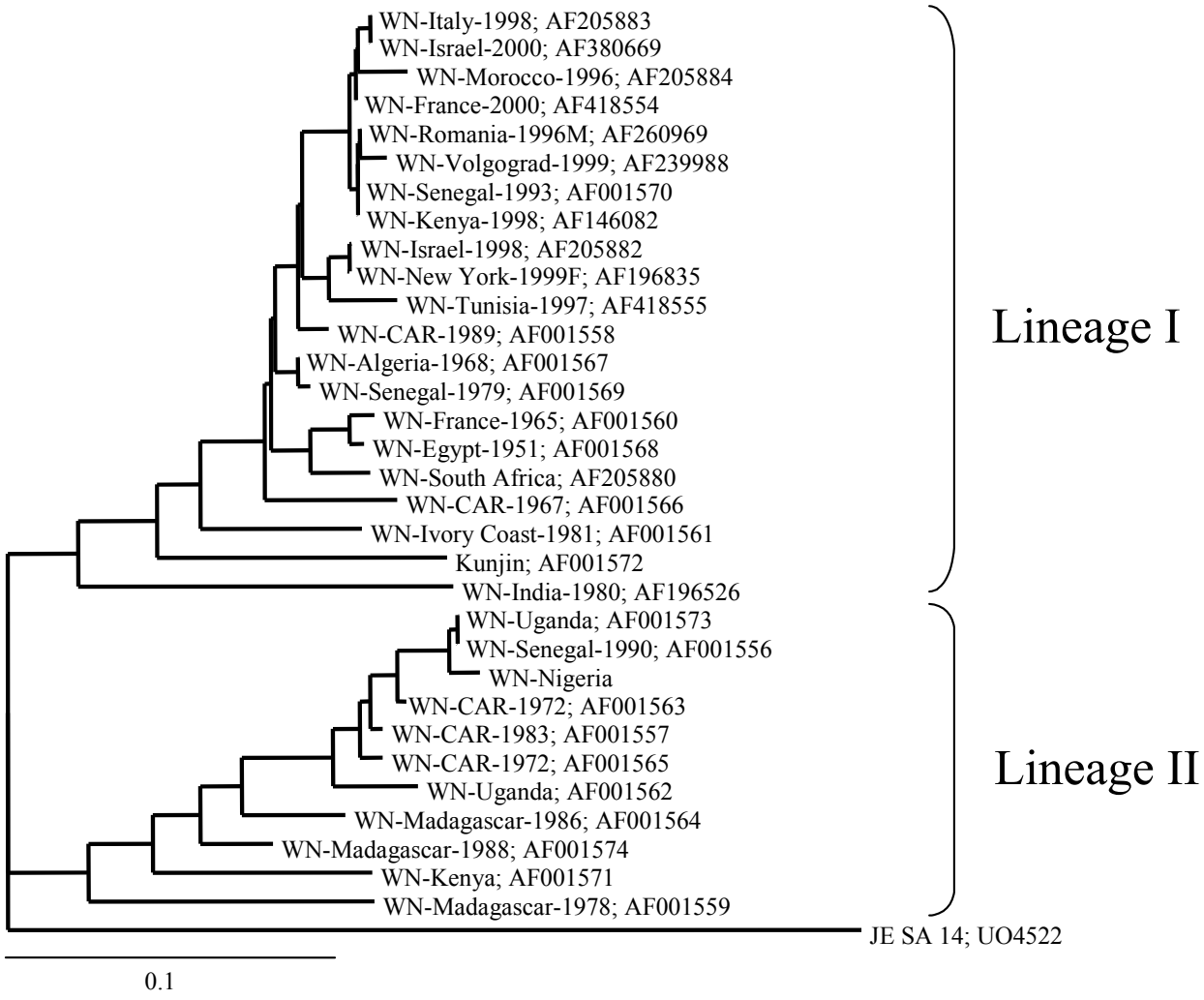
The main hosts of WNV are birds, humans and equines (mainly horses). However, WNV has been detected in several animals such as cats, dogs, camels, bats, chipmunks, squirrels, skunks, rabbits, harbour-seals, raptors and alligators.

In North America WNV has been detected in dead birds of at least 138 species. Although birds, particularly crows and jays infected with WNV can die or become ill, most infected birds do survive. In Europe bird mortality related to WNV infection has not been reported. However, in Israel several storks and geese died from WNV infection.

4.3. Transmission cycle

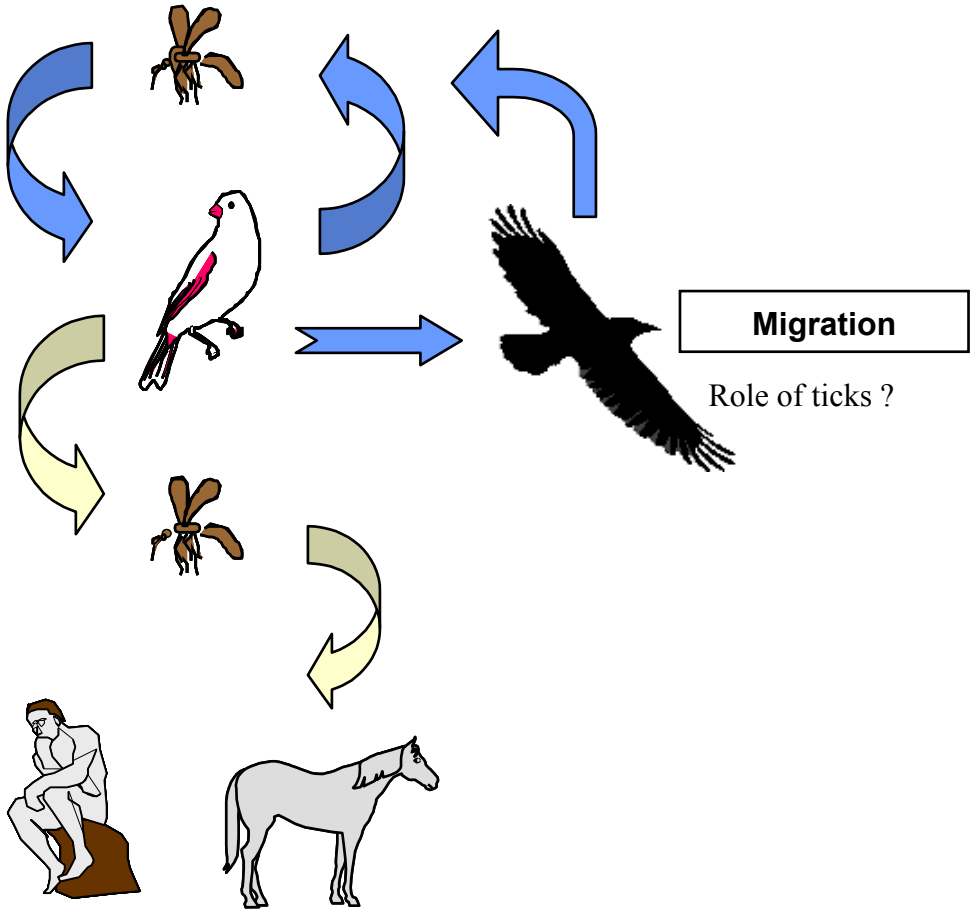
WNV occurs in a complex life cycle involving a non-human primary vertebrate host (usually bird) and a primary mosquito vector. WNV is amplified during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and birds (Figure 2). Infectious mosquitoes carry virus particles in their salivary glands and infect susceptible bird species during blood-meal feeding. Immunocompetent birds will sustain an infectious viraemia (virus circulating in the bloodstream) for 1 to 4 days after exposure, after which these hosts develop life-long immunity. Humans, horses, and most other mammals are incidental or "dead-end" hosts because they do not produce significant viraemia, and do not contribute to the transmission cycle**.**

Figure 1. Phylogenetic tree based on nucleic sequence data of WN virus E-protein gene fragment of 254bp (Murgue *et al.*, 2002)



Genebank accession numbers for the sequences included in the tree are indicated.

Figure 2. West Nile virus – Transmission cycle



However, the transmission cycle of WNV is rather complex and not fully understood and the possible role of ticks in the transmission has been discussed. The transmission cycle implies a chain of events that could allow the amplification of the virus and, in some unknown circumstances, its further transmission to mammals. Many factors have been suggested to be involved in the occurrence of WNV in mammals, including density of bird and mosquito populations, land cultivation, climatic conditions, etc.. However, in Europe, WNV outbreaks are erratic and spatio-temporary limited phenomena, occurring quite unpredictably, even if all conditions appear to be present in a definite place.

4.4. Human infection

The principal route of human infection with WNV is through the bite of an infected mosquito. Additional routes of infection have become apparent during the 2002 WNV epidemic in USA. WNV transmission through transplanted organs and blood transfusion, as well as transplacental (mother-to-child) transmission, have been documented.

Most people who become infected with WNV do not suffer any illness. It is estimated that 10 to 20% of people who become infected will develop WNV fever after an incubation period of 3 to 14 days: mild symptoms, including fever, headache, and body aches may occur, occasionally with a skin rash on the trunk of the body and swollen lymph glands.

The symptoms of severe infection (WNV encephalitis or meningitis) include headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis. It has been estimated that 1 in 150 persons (0.6%) infected with WNV will develop a more severe form of disease, possibly with a fatal outcome. Other severe forms of the disease have been described such as acute pancreatitis and fulminant hepatitis.

Symptoms of mild disease generally last a few days. Symptoms of severe disease may last several weeks, and neurological complications may be permanent.

There is no specific treatment for WNV infection. In more severe cases, intensive supportive therapy is indicated, often involving hospitalisation, intravenous fluids, respiratory support (ventilator), prevention of secondary infections (pneumonia, urinary tract infection, etc.), and good nursing care.

4.5. Outbreaks of WNV infection

4.5.1. WNV 1951 – 1994

The improved knowledge about WNV infection came from studies performed in Egypt. Indeed, the discovery in July 1950 of WNV virus in the blood of 3 children and WNV antibodies in a high percentage of the inhabitants in a village located in the north of Cairo, led the United States-Naval Medical Research Unit (US-NAMRU) to conduct a four year programme, beginning in the autumn of 1951, to study WNV and its epidemiology (Taylor *et al*., 1956).

The investigations were mainly in a restricted area in the upper Nile Delta, including (i) serological surveys in humans, mammals and birds, (ii) isolation of virus from naturally-infected hosts and vectors, and (iii) experimental infection in humans, equines, birds (mainly crows and sparrows) and mosquitoes (*Culex* species). Overall, the results demonstrated that the main cycle of the virus involved mosquitoes and birds, in which humans and equines could be infected; but are considered to be dead end hosts.

The ecological studies demonstrated in a specific area that the percentage of humans with WNV antibodies was correlated with the percentage of crows with antibodies to WNV. This observation led to the concept of non-endemic, transitional and endemic zones (Work *et al*., 1955). Examination of the environment in these zones revealed differences which, in combination, could account for the persistence and activity of the virus, e.g. differences in the density of the human population and the intensity of land cultivation, and differences in the prevalence of mosquitoes and birds. In contrast, climatic conditions did not appear to be a significant factor. The persistence of WNV during the 3-year study in Egypt was explained by mosquitoes that remained

active throughout the colder months. However, during this 3-year study period, no WNV epidemic was reported.

The first real epidemic in the region was described in Israel in 1951, with 123 cases and no death. The first severe cases were reported in 1957 in Israel. WNV was subsequently responsible for an outbreak in France in 1962-1963, during which about 80 horses presented neurological disorders characterised by ataxia and weakness. Twenty five to thirty percent of them died and several human cases of encephalitis were also reported.

Between 1960's and 1980's, WNV was isolated from mosquitoes, birds and mammals in several European countries (Spain, Portugal, Romania, Czech Republic, Slovakia, Poland and Russia), as well as in Africa, the Middle East and India. During that period, sporadic cases, including severe cases (such as encephalitis and acute hepatitis), were reported in Africa and India. However, apart from the 1974 epidemic in South Africa, where there was an estimate of 18,000 WNV human infections (no deaths), no major outbreak occurred. Thus WNV infection was not considered to be a public health problem, although the dispersion of the virus covered all the Old World, except Southeast Asia (Murgue *et al.*, 2002).

4.5.2. Recent outbreaks 1994 - 2002

Since 1994, WNV has appeared to be a re-emerging virus, with several outbreaks in the Mediterranean region and Eastern Europe and with detection of WNV for the first time in America in 1999. The 1996 epidemic in Bucharest was the first to occur predominantly in an urban setting. A short description of these outbreaks follows.

4.5.3. Main outbreaks in EU member states

Italy 1998

In Italy, 14 horses located in Tuscany were laboratory-confirmed to be infected with WNV between August and early October 1998, of which 6 animals died (Cantile *et al.*, 2000). WNV was isolated from a brain biopsy. All animals presented with neurological signs for 2 to 15 days. Histologically, all dead animals showed non-suppurative slight to moderate encephalomyelitis, with lesions predominantly observed in the spinal cord and the lower brain stem. Neither serological surveys in humans or horses, nor entomological investigations are available.

France 2000

In Southern France in 2000, 76 equines were laboratory-confirmed with WNV from 131 equines presenting with neurological disorders between September 6 and November 30, including 21 fatal infections. The last confirmed case was on November 3. WNV was isolated from a horse brain biopsy. All but three cases were located in a region called "la petite Camargue" (Herault and Gard provinces) harbouring several large ponds, numerous colonies of migratory and settled birds, as well as large populations of mosquitoes.

No human cases were laboratory-confirmed among 51 suspected cases including 33 cases hospitalised with symptoms of encephalitis or meningoencephalitis, and 18 other cases with fever. In contrast, WNV antibodies were detected by neutralisation tests in 2 of 33 gamekeepers working in this area, one had a history of travelling in tropical countries, but the other, who had a low but detectable IgM antibody level, had not travelled at all. Thus, although human transmission occurred during this outbreak, it was impossible to evaluate the level of human infection among persons living in the infected area without undertaking a serological survey (Murgue *et al.*, 2001).

A serological survey was undertaken in horses located within a radius of 10 km around the confirmed cases. A total of 5,133 samples of horse sera were collected between September and November 2000 from the three different provinces where cases had been reported. WNV ELISA IgG was positive for 428 (8.3%) of these samples of which 248 (41.4%) were also IgM positive. There was a direct relationship between the number of positive clinical cases in an area, and the number of positive cases included in the serological survey study (Durand *et al.*, 2002).

No abnormal mortality was reported in birds. WNV-neutralising antibodies were found in some sera of birds collected in November and December 2000.

4.5.4. Main outbreaks in Central European countries

Romania 1996

Between July 15 and October 12, 1996, 835 patients were hospitalised with suspected central nervous system infection. Appropriate blood samples were available for 509 of the patients, of whom 393 (77%) were laboratory confirmed to have antibodies to WNV, including 286 from Bucharest. Other positive cases were located in nearby rural areas. There were 17 fatalities in patients older than 50 years. A serological survey study was performed on 959 Bucharest residents, which found that 4% of the inhabitants were IgG positive (Tsai *et al.*, 1998). Virus was isolated from brain tissue samples of patients at autopsy.

Entomological and avian investigations conducted in October 1996 (Savage *et al.*, 1999) demonstrated the presence of neutralizing antibodies to WNV in 41% of the 73 domestic fowl tested (ducks, chickens, and turkeys) and in 1 of 12 Passeriformes tested. Among 5,577 mosquitoes collected (mainly *C. pipiens pipiens*) only one WNV isolate was obtained from a pool located near the centre of Bucharest (infection rate of 0.19 per 1,000). This low infection rate might be due to the delay in starting the investigations, several months after the outbreak. A risk factor study demonstrated that increased exposure to the mosquito vector, particularly in the home, was associated with risk of acquiring WNV infection.

During 1997 and 1998, neurological infections were diagnosed serologically as WNV encephalitis in 12 of 322 and 1 of 75 patients, respectively. There was one fatal case in 1998 (Cernescu *et al.*, 2000).

In 1999, among 686 infections with CNS involvement, WNV was confirmed for 7 of them, including one fatal case, and in 2000, 13 human cases were laboratory-confirmed including 2 deaths (Ceianu C., personal comm.)

Russia 1999

A large outbreak of severe encephalitic disease occurred in the Volgograd region in Russia between August and September 1999 (Platonov *et al*., 2001). Among 826 patients admitted to hospital during this period with the clinical diagnosis of acute aseptic meningo-encephalitis, serum samples were tested from 318 patients of whom 183 (58%) were laboratory confirmed to have been infected with WNV. The total number of suspected overt human WNV cases was estimated to be 480. Most of the patients (85%) were from Volgograd and Volzskii cities, the rest were from rural areas around Volgograd. The disease was severe as 40 fatal cases were reported (75% older than 60). Virus was isolated from brain tissue samples.

4.5.5. Main outbreaks in North African countries

Algeria 1994

In Algeria, an epidemic occurred between August and September 1994 in Timimoun oasis in central Sahara. About 50 cases presented with high fever and neurological signs and among them, 20 were clinical cases of encephalitis of whom 8 died. WNV serology on 18 cases (14 clinical cases and 4 probable) revealed 17 of them were found to be positive for the virus. All the 14 clinical cases were IgM-positive and 13, were children of 10 months to 9 years (Le Guenno, 1996).

Morocco 1996

Between August and mid October 1996, 94 equines were affected in Morocco (in the provinces of Kenitra and Larache), 42 of which died, and the disease was reported in all age categories (Tber Abdelhaq *et al.*, 1997). Virus was isolated from a brain biopsy. A human encephalitis case was also suspected to be due to WNV.

Tunisia 1997

In Tunisia, 173 patients were hospitalised for meningitis or meningoencephalitis in two coastal districts, Sfax and Mahdia, between September 7 and December 12, 1997, and 8 patients died. The epidemic peak was reached between the last week of October and the second week of November. Among 129 patients tested, 111 cases were WNV IgM ELISA positive (86%) including 5 fatal cases (4.5%). Among the positive cases, WNV IgM ELISA was performed on the CSF of 23 patients and was positive for 9 cases including 6 (3 fatal cases) for whom only CSF could be obtained (Murgue *et al.*, 2002). Virus was isolated from brain tissue samples.

4.5.6. Main outbreaks in Middle East countries

Israel 1998-2000

In 1998, 18 serum samples from horses suffering from encephalomyelitis were found to have WNV neutralising antibodies, and virus was isolated from the brain of a stork (Malkinson *et al.*, 1998). In 1999, thousands of geese were destroyed when WNV was identified in commercial flocks (Malkinson and Banet, 2002). A high genomic similarity was found between a WNV strain isolated from the brain of a dead goose in 1998 and the 1999 New-York isolate.

Two fatal human cases were reported in 1999 in a suburb of Tel Aviv and the WNV strain isolated from the brain of one patient was nearly identical to the avian strain of 1998.

From August to October 2000, 417 laboratory-confirmed WNV fever cases occurred in humans, of whom 325 were hospitalised in the northern and central parts of Israel. Data were collected from 233 of these patients (median age 65 years, ranging from 3 to 95 years) of whom 33 (14.1%) died. All fatal cases but one were $\geq$ 68 years (median age 80 years, ranging from 54 to 95). WNV was isolated from serum samples of four patients. Sequencing and phylogenetic analysis revealed co-circulation of two genetic variants, one closely related to the Israel 1998 and New York 1999 isolates, and the other one to the 1999 Russian isolate (Weinberger *et al.*, 2001)

4.5.7. Main outbreaks in North America

USA, Canada and the Caribbean 1999 – 2002

In September 1999, WNV was detected for the first time on the American continent, in New York. The outbreak was responsible of 62 human encephalitis cases (including 7 deaths), and 20 equine encephalitis cases (9 deaths).

This outbreak was remarkable for many reasons, but especially because of the mortality in infected birds (mainly in crows and blue jay), which facilitate dispersion of the virus. A consequence of this dispersion was that, in 1999, WNV was detected in New Jersey and 3 surrounding States.

The virus persisted during the 1999-2000 winter, and from August to October 2000, WNV was present in 12 States, responsible for 8 human and 60 equine encephalitis cases, as well as thousands of dead birds.

Subsequently, the virus spread further in the USA (20 States in 2001 and 39 States in 2002), Canada and even to the Caribbean. In October 2001 in the Cayman Islands, a case of WNV infection was detected in a patient that had not travelled outside the island during the previous 6 months. In 2002, WNV was responsible for 3,893 human cases (254 deaths), 14,717 equine cases (4,500 deaths) and more than 13,000 deaths in birds.

The North-American example shows that WNV finds an ecological niche that allows it to survive during the winter and to spread progressively. The bird mortality allows epidemiologists to follow the spread of the virus and thus represents a good sentinel. It can be expected that WNV will spread further North (Canada) and South (Caribbean and Central and South

America) following a scheme of dissemination that probably happened in the Old World in the past.

Particular concern about WNV transmission via blood and cell transfusion (Biggerstaff and Petersen, 2002; MMWR, 2002a,c) and organ transplantation, as well as evidence of laboratory acquired WNV infection (MMWR, 2002d), and possible risk via breast feeding (MMWR, 2002d) led to specific research plans by the CDC, FDA, and pharmaceutical industry on "model viruses", testing a variety of inactivation procedures, which concluded that WNV did not behave differently from other flaviviruses and was inactivated using the same procedures. Information about WNV and blood safety was given by the FDA (2003). Recommendations for donor deferral and procedures of inactivation of the virus in labile and stable blood products are given in details in the proceedings of the FDA/CDC workshop on WNV, November 2002.

5. DIAGNOSTIC TOOLS

WNV infection can be detected by virus isolation (or virus genome detection) or by IgM (or IgG) antibody detection in serum and acute cerebrospinal fluid (CSF). Testing includes WNV-reactive IgM and IgG by ELISA, polymerase chain reaction (PCR) and plaque-reduction neutralisation (PRN) tests (Petersen *et al.*, 2002). WNV laboratory diagnosis can be performed from the following biological samples:

- serum and CSF for antibody testing

- tissues (especially brain and spinal cord, CSF, blood or other body fluids) for virus/genome detection.

5.1. Virological laboratory diagnosis:

The experience gained in WNV diagnostic testing over the past 3 years has led to the following observations:

5.1.1. Virus isolation

Attempts to isolate WNV can be made in known susceptible mammalian (Vero, RK13 cells in particular) or mosquito cell lines. Cells originating from mosquitoes may not show cytopathic effects but can be screened by immunofluorescence.

Tissues

When arboviral encephalitis is suspected, the following tissues can be sampled: brain (including various regions of the cortex, midbrain and brainstem), blood and CSF, even if the tissues are grossly decomposed. These samples have to be tested in specialised laboratories for arboviruses. Available techniques include gross pathology, histopathology, RT-PCR tests (Polimerase Chain Reaction using Reverse-Transcriptase), virus isolation, and immunohistochemistry. RT-PCR can be used to rapidly detect WNV RNA in tissues.

Appropriate samples for virus isolation are prioritised as follows:

- Clinically ill humans: CSF; serum samples may be useful early in infection,

- Humans (biopsy or post-mortem): brain tissue (especially brain and spinal cord),

- Horses: serum and CSF for antibody testing,

-Horses (post-mortem): brain tissue (including brainstem), spinal cord tissue,

- Birds: kidney, brain, heart,

- Other mammals: multiple tissues, especially kidney and brain.

Virus isolate identity can be accomplished by indirect immunofluorescence assay (IFA), using virus-specific monoclonal antibodies, by nucleic acid detection or by virus neutralisation. The IFA using well-defined murine monoclonal antibodies (MAbs) is the most efficient, economical, and rapid method to identify flaviviruses. MAbs can differentiate WNV from St. Louis encephalitis virus and from other flaviviruses. Flavivirus-grouping MAbs are available for use as positive controls. Nucleic acid detection methods including RT-PCR, TaqMan and nucleic acid sequence based amplification (NASBA) methods may be used to confirm virus isolates as WNV.

5.1.2. Virus Detection in Tissues

Immunohistochemistry (IHC) using virus specific MAbs on brain tissue is very useful in identifying both human and avian cases of WNV infection. In suspected fatal cases, IHC should be performed on formalin-fixed autopsy, biopsy, and necropsy material, ideally collected from multiple anatomical regions of the brain, including brainstem, midbrain, and cortex.

A well-characterised antigen-capture ELISA is available for the detection of WNV and SLE virus antigen in mosquito pools and avian tissues.

5.1.3. Nucleic acid analysis

RT-PCR of tissues, CSF, and serum has proved to be a reliable method in avian, equine and human surveillance. A number of nucleic acid detection methods have recently been employed for WNV diagnostic and surveillance purposes. Fluorogenic 5' nuclease techniques (real-time PCR) and nucleic acid sequence based amplification (NASBA) methods have been developed and undergone initial validation in specific diagnostic applications.

5.2. Serological laboratory diagnosis

The most efficient diagnostic method is detection of IgM antibody to WNV in serum or CSF. The IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) is optimal for IgM detection because it is simple, sensitive and applicable to serum and CSF samples. However, as ELISA for WNV may cross-react with other flaviviruses (e.g. St. Louis encephalitis,

dengue, Yellow Fever) it should be viewed as a screening test only. Initial serologically positive samples should be confirmed by neutralisation test if collected in regions where different flaviviruses circulate. Among the patients in New York City, infected in 1999 and 2000 and for whom a sample of CSF was available, nearly all (95%) had demonstrable IgM antibody. Since IgM antibody does not cross the blood–brain barrier, IgM antibody in CSF strongly suggests central nervous system infection. Ninety percent of serum samples obtained within 8 days of symptom onset were also positive for IgM antibody. Similar results have been reported during the 1997 outbreak in Tunisia. Tests of serum or CSF are available commercially and can be obtained through local, state, or province health departments for patients with encephalitis or meningitis (Petersen *et al.*, 2002).

There are two caveats when interpreting serological tests.

Firstly, due to close antigenic relationships among the flaviviruses, individuals recently vaccinated with yellow fever or Japanese encephalitis vaccines or recently infected with a related flavivirus (for example, St. Louis encephalitis or dengue) may give positive results on IgM antibody tests for WNV. The plaque reduction neutralisation test, the most specific test for the arthropod-borne flaviviruses, can be used to help distinguish false-positive results on IgM antibody-capture enzyme-linked immunosorbent assay or other assays (for example, indirect immunofluorescence and hemagglutination inhibition). The plaque reduction neutralisation test may also help distinguish serologic cross-reactions among flaviviruses, although some degree of cross-reaction in neutralising antibody may still cause ambiguous results.

Secondly, since most infected persons are asymptomatic and because IgM antibodies may persist for 6 months or longer, residents in endemic areas may have persistent IgM antibody from a previous infection unrelated to their current clinical illness.

In horses, it has been shown that IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) is also a useful technique for WNV diagnosis (Bunning *et al.*, 2002; Murgue *et al.*, 2001)

Serum

An increase in WNV specific neutralising antibody titers in serum specimens from persons with acute and convalescent disease confirms acute infection (Petersen *et al.*, 2002). Paired acute-phase (collected 0-8 days after onset of illness) and convalescent phase (collected 14-21 days after the acute specimen) serum specimens are thus useful for demonstration of seroconversion to WNV and other arboviruses by ELISA or neutralization tests. The detection of IgM by ELISA provides evidence of an early infection.

Cerebrospinal fluid

As early as the first few days of illness, IgM antibodies to WNV can be demonstrated in CSF by antibody-capture ELISA (Enzyme Linked Immuno-absorbent Assay).

6. ANIMALS TO BE USED AS SENTINELS

6.1. Equidae

6.1.1. WNV infection in horses

Horses become infected with WNV after being bitten by an infected mosquito. Once a horse has been bitten, it may take only 5 to 15 days for clinical signs of WNV to appear. Horses infected with WNV have very low viraemia titers, so they cannot transmit the virus to other mosquitoes. This has recently been confirmed by experimental infections in horses (Bunning *et al.*, 2002)

The majority of horses exposed to WNV do not become ill. During the sero-survey conducted in France in 2000, of 5,133 horses tested, 4.8% were IgM postive in the absence of clinical symptoms (Murgue *et al.*, 2002)**.** Horses become ill and show symptoms when the virus crosses the blood-brain barrier and causes inflammation of the brain (encephalitis) and/or the spinal cord (myelitis). Symptoms of WNV infection include depression and appetite loss, along with one or more of the following: ataxia/stumbling; fever; weakness; paralysis in the hind legs; muzzle twitching; impaired vision; head pressing; circling; aimless wandering

Other neurological diseases can be confused with WNV. The following diseases can induce identical clinical signs: equine protozoal myeloencephalitis (EPM); rabies; Eastern, Western and Venezuelan equine encephalomyelitis (EEE, WEE, VEE); cervical vertebral myelopathy (CVM, wobbler syndroms); equine degenerative myelopathy (EDM); equine herpes virus 1 (EHV-1); and Borna disease.

6.1.2. Suitability of horses as sentinels

The surveillance of WNV can be performed using a passive surveillance system (based on clinical diagnosis of WNV infection in horses) or by active surveillance (development of a specific epidemiological network for prospective WNV and/or WNV-antibody detection). Horses are good sentinels for WNV infection surveillance. Horses are easily identifiable, individually or by herds. The role of horses in the epidemiological cycle of WNV, dead-end hosts like humans, the facility to capture and sample horses, the availability of serological tools (IgM antibody-capture enzyme-linked immunosorbent assay), the possibility of combining WNV detection and detection of other types of diseases in horses, the veterinary network and the implication of the public veterinary services in most countries, are among the reasons to consider horses as good sentinels if a WNV surveillance system is to be organised.

6.1.3. Passive surveillance

The combination of clinical observations with diagnostic test results allows veterinarians to accurately identify cases of WNV encephalitis in horses. In the majority of European countries, horses that show WNV-like clinical signs (neurological disorders), e.g. in France in 2000 (Murgue *et al.*, 2001). The surveillance case definition for WNV encephalitis in equine must be as

sensitive as possible, yet minimise false-positive case classifications. One of the reasons for performing surveillance for equine WNV encephalitis is to meet international obligations for disease reporting. Such disease reports can have substantial consequences for the international movement of horses and other livestock. A high level of specificity in case classification is therefore critical, especially when detecting and reporting the first case of disease in a given geographic area (e.g. a previously unaffected country or region). Given the specificity of the case definition, failure of a clinically ill equine to meet the criteria for a probable or confirmed case does not completely exclude the possibility that WNV was the cause of illness.

In each European country, reference laboratories have the ability to achieve virological and/or serological diagnoses. In EU Member States Equine encephalomyelitis of all types (including WNV), are compulsory notifiable diseases, under control of the Official Veterinary Services. Various regulatory measures should be available to facilitate the best diagnosis possible. If WNV is introduced into a free-European region, this passive surveillance system would alert the Public Health authorities and allow epidemiologists to follow the evolution of the infection. It must, however be noted that the efficacy of the "passive horse surveillance system" depends on the morbidity induced by the various WNV strains.

6.1.4. Active surveillance

The presence or absence of WNV circulation can be monitored prospectively by following the serological status of a horse population, as was achieved in France (Perra *et al.*, 2002). Horses followed as sentinels are sampled regularly to detect specific sero-conversion, and must be stabled in the area where the surveillance has to be performed. This active surveillance seems particularly indicated in those areas where outbreaks have occurred and/or in areas considered especially at risk because of their climatic and/or land use characteristics (river deltas, frequently flooded areas, areas rich in ponds, lakes and/or marshes, etc.).

However, despite the advantages of focusing the screening on horses, active surveillance is an expensive and time-consuming system that necessitates a high concern by the owners and an extensive collaboration between the different partners involved.

6.2. Birds

The first isolations of WNV from avian species were reported by Work *et al*. (1953) from a hooded crow and rock pigeons in the Nile delta region. Subsequently WNV has been shown to infect a large number of different bird species and their role as potential reservoirs for the virus has long been recognised (Work *et al*., 1955; Taylor *et al*., 1956). More recently, the role of birds in the ecology of WNV in Europe and Africa has been reviewed by Malkinson and Banet (2002). The effects of WNV infection on birds varies considerably between species, from a fatal disease to a mild or even a sub-clinical infection.

Due to their role in introducing and spreading WNV, birds have always been considered useful candidates as sentinels for the presence of the virus in a

geographical location. Komar (2001) reviewed the various strategies for WNV surveillance using birds or specific bird species as indicators of the presence of the virus that had been deployed in various areas of the world. Essentially these may be divided into four groups 1.) dead wild birds; 2.) captive sentinel birds; 3.) trapped wild birds; 4) domestic poultry.

6.2.1. Dead wild birds

At its most basic level, passive surveillance determines increased mortality in susceptible bird species in which the infecting virus strain causes death: such as the North American WNV strain in American crow (*Corvus brachrynchos*) and other corvids alone may be used as an indicator of WNV activity (Eidson, 2001). However, more usually surveillance of dead bird has involved sampling for virus isolation and/or detection. This imposes an active element into the surveillance strategy as different species of bird can be targeted relating to susceptibility and, for example, whether they represent migratory or resident species.

Outside North America and Israel WNV has not been reported to cause high mortality in infected birds and examination may be limited to birds dying from other causes. In some countries, such as the UK, WNV susceptible resident birds such as Corvidae species are regarded as a pest and often culled, and they may be a useful source for surveillance studies.

6.2.2. Captive sentinel birds

The use of captive sentinel birds for detecting WNV has been reviewed in detail by Komar (2001). Essentially this involves keeping captive susceptible birds at the desired location and testing them for antibodies to WNV at regular intervals. As stated by Komar (2001), the ideal sentinel bird would be susceptible to infection, resistant to disease, present negligible health risk to handlers, and not contribute to local transmission. Komar (2001) further states that there is probably no ideal sentinel bird. Although several captive species of birds, such as pheasants, pigeons and even trapped wild birds have been used as sentinels, caged domestic fowl are usually the bird of choice and have been used throughout the USA (Komar, 2001), as well as in South Africa (McIntosh *et al.*, 1967), Australia, for Kunjin virus, (Russell, 1998) and Romania (Cernescu *et al.*, 2000). This is because they develop measurable levels of antibodies quickly after infection and do not usually show clinical signs. The virus does not spread from chicken to chicken, nor to the handlers or to mosquitoes. The usefulness of chickens as sentinel birds has been controversial. In Romania in 1997 sero-conversion occurred in 40% of the sentinel chickens and was detected up to six weeks before WNV infections of humans. However, in New York and New Jersey, for example, sero-conversion rates in sentinel chickens were much lower 1.4- 7.9%, and occurred after the onset of the first human case (Cherry *et al.*, 2001; Komar, 2001).

Important considerations in the use of captive sentinel birds, in addition to the species chosen, are location, type and positioning of cage, the number of birds and any other factor that may render the sentinels more or less attractive to the vector.

6.2.3. Trapped wild birds

WNV has been shown to infect a very large number of both migratory and resident wild bird species (Malkinson and Banet, 2002) and, as with other arboviruses, testing of trapped wild birds for antibodies has been used to detect virus activity in a geographical area. Point prevalence surveillance in wild birds is probably only useful when young birds hatched that year are surveyed. Usually, surveillance using wild birds has been to trap, bleed and leg-band the individual birds before releasing and then examine for changes in antibody titre when, the same bird is trapped at a later date. As discussed by Komar (2001), in addition to the fact that most released birds will not subsequently re-trapped, this surveillance strategy poses several problems such as selecting species to be sampled, determining trapping sites, distinguishing between true resident and migratory species, determining the age of the trapped bird, and other factors including the serological test to be used.

6.2.4. Domestic poultry

In many respects domestic poultry, especially chickens, represent a useful target animal for WNV surveillance and overcome many of the problems associated with wild birds. Their age is known, their initial serological status can easily be checked, they are generally kept in large flocks and may be widespread in a given geographical area, small backyard flocks may even be found in urban areas. In Israel young domestic geese showed high morbidity and mortality in natural infections with WNV and outbreaks occurred each year from 1997-2000 (Malkinson and Banet, 2002) and could represent a sentinel species.

6.3. Mosquitoes

WNV has been isolated from a large number of mosquito species of several genera. However, the collection of mosquito is not a practical method for routine surveillance of transmission, since the proportion of mosquito pools in wild populations that test positive is very low, even when transmission rates are high. During an intensive study in Egypt in 1951-1954 WNV was only isolated in 1.7% of mosquito pools tested. Isolation rates have been similar in the United States, despite the use of the Gravid *Culex* Trap, a device that selectively captures large numbers gravid *Culex pipiens* and *Cx. restuans* (the principal avian vectors in many parts of the country) i.e. older females that have taken at least one blood meal.

6.4. Other animals

Based on present knowledge, no other species appears to be a good candidate.

7. SURVEILLANCE AND MONITORING SYSTEMS CARRIED OUT IN EUROPE

7.1. France

The results of the WNV surveillance system in France (Perra *et al.*, 2002) are summarized in Table 1. This surveillance system was conducted as follows:

Human:

In 2001 and 2002, those patients who presented neurological disorders (encephalitis signs and symptoms), were tested for serological diagnosis of WNV infection.

Mosquitoes:

In 2001, mosquitoes were trapped in the South of France. About 20 traps were regularly screened; mosquitoes were selected from the pools of insects and the WNV genome RNA was detected by RT-PCR. Regarding the low cost/benefit of such a system, the "mosquito surveillance" was no longer used in 2002.

Birds:

In 2001, 184 domestic ducks and 142 domestic chickens were regularly sampled (every four weeks from May to November 2001). In 2002, 150 domestic ducks were used as sentinels and sampled every three weeks.

Horses:

In 2001, 30 clinically suspected horses were tested against WNV (passive surveillance). In 2002, active surveillance was carried out; 120 sero-negative horses were used as sentinels and serologically monitored during summer 2002.

The WNV surveillance system carried out in France in 2001 and 2002 has necessitated the involvement of different partners (especially Public Health institutions, National Veterinary Services and National Veterinary and Human Laboratories) which have also worked together to warn the general public.

Table 1. Results of the surveillance system established in France in 2001-2002.

	Human positive/tested	Horses positive/tested	Birds positive/tested	Mosquitoes positive/tested
2001	0 / 19	0 / 30	1 / 326	0 997 pools
2002	0 / 16	0 / 18 (passive) 1 / 120 (active)	1 / 150	ND

ND: not done

7.2. Spain

The Spanish surveillance system is based on the notification of suspected human clinical cases (no confirmed cases to April 2003). A survey of mosquitoes has been carried out and none of the pools tested was positive. However, in 1996, to check for the prevalence of the WNV infection and other viruses transmitted similarly among the human population of the Ebro Delta, 1,037 samples of serum taken in November 1980 in 10 to WNV of this area were analysed for the presence of WNV antibodies and antibodies against other 12 arthropod-borne viruses (3 *Alphaviruses*, 8 *Flaviviridae* and 1 *Bunyaviridae*) by inhibition of haemagglutination (HAI). In some cases, the presence of HAI-specific IgM was analysed. In all, a significant degree of reaction was found to some of the viruses tested in 130 cases (12.5% overall; 4.1% to *Alphavirus*; 8% to *Flaviviridae* and 0.4% to *Bunyaviridae*). The analysis of the antibody titres revealed significant numbers of samples showing high titres to WNV and other types of viral antigens. The distribution of the antibody prevalence was very uneven, being focused mainly in 3 localities located in land on the Delta (Ampolla, San Jaime and Montells), where the prevalence of *Flaviviridae* antibodies was as high as 30%. Residual levels of WNV-related IgM were found in some serum samples (Lozano and Filipe, 1998). Preliminary data from a serum survey in 2002, in the same area, indicate that 8/797 (1%) were positive for WNV antibodies (Antonio Tenorio, for the EVITAR network, personal communication).

7.3. Italy

A surveillance programme has been implemented in 2002, in risk areas selected on the basis of climatic and ecological factors and the presence of vectors. The main activities are 1) sampling in sentinel chicken flocks located in the study areas, every 15 days; 2) sampling wild birds belonging to susceptible species in the study areas; 3) collecting serum in horses in the study areas; 4) surveying mosquitoes, using CDC traps, in the same areas.

In the case of detection of WNV in at least one of the Italian study areas, definition of vector competence and vector capacity of each species of potential vectors should be assessed. Maps of WNV infection distribution and of the distribution of each relevant species of potential vectors will be established for each study area.

7.4. Romania

Following the 1996 epidemic, the surveillance system was mainly based on a passive hospital-based surveillance of human encephalitis. This allowed detection of sporadic cases each year in districts neighbouring the Danube, with a cluster of 7 cases in Bucharest in 2001. A national reference laboratory in the Cantacuzino Institute, Bucharest, is responsible for all confirmations in humans, and also for mosquito and bird studies. Community-based control programs using insecticides (including larvicide) was established since, at least in Bucharest. The general public is warned each year about the specific preventive measures against mosquitoes.

7.5. Great Britain

Birds

Formal surveillance of birds was started by the Veterinary Laboratories Agency (VLA) in 2002. Currently this consists of examining dead wild birds submitted by VLA Regional Laboratories throughout England and Wales for the presence of WNV using virus isolation and PCR. During 2002 approximately 300 bird carcases were processed and all were negative. There are plans to upgrade this surveillance during 2003. This will be specifically to target known susceptible species, especially *Corvidae* species, bird 'die offs', and areas of known high mosquito activity.

Mosquitoes

There is no formal surveillance at present, but plans have been made to begin surveillance by collecting mosquitoes in targeted sites and screening for WNV by PCR.

Horses

WNV is routinely considered as a differential diagnosis in horses showing encephalitis, but there has been no formal surveillance to date. Surveillance is planned based on serology, targeting free range feral herds.

Humans

Retrospective studies and enhanced awareness of possible WNV infections in 2002 failed to demonstrate that any patient with encephalitis or meningitis of undetermined cause was infected with WNV.

7.6. Nordic countries

No active surveillance in animals is performed in Nordic countries. However, activities are in place in order to detect WNV in suspected human or animal meningo-encephalitis cases.

8. ADVANTAGES AND DISADVANTAGES OF A SURVEILLANCE PROGRAMME

The objectives of a surveillance programme are:

- to detect the risk of the virus transmission to the human population (likely in relation to transmission cycle amplification)

- to detect the first human and/or animal case in order to prepare the components of the human and veterinary public health systems for a more intensive surveillance

- to follow the evolution of an outbreak

Proactive surveillance programmes and mass screenings are usually considered when a specific action may be implemented to prevent and/or cure the disease in humans. This type of surveillance, which mobilises a high number of components and resources, seems justified if the identification of the infectious agent is reliable, if human or animal sentinels are available, in those areas where large populations of humans are involved, where severe forms of the diseases are observed, where fatalities are high, and if appropriate measures lead to a better management of the outbreaks. It may be noted that, for WNV infection in humans, no specific prevention measures, except usual non-specific measures of protection against mosquito bites, and nonspecific treatment, are currently available. Whatever the aetiological infectious agent, except for a limited number of viruses, patient's management of severe cases of meningitis and/or encephalitis includes non-specific measures and these cases are not accessible to any specific treatment. An alert system would, however, help physicians to correctly identify the cases and to prevent inter-human dissemination via transfusion or transplantation, and environmentalists to act on mosquitoes and on animal reservoirs.

Emergence of WNV disease in the USA, with nearly 4,000 confirmed human cases and more than 250 deaths, as well as the high number of human cases with symptomatic diseases and the involvement of urban populations in central Europe countries, within the past decade, have made WNV a concern for Public Health, and raised the question of a systematic surveillance of the disease in animals and in humans. Such surveillance, which appears, in this instance, justified, would involve, at least, a Public Health sentinel network to disclose human new cases, and a complex network of public institutions and veterinarians to disclose the disease or its markers in the main animal reservoir and in animals that occupy the same place as humans in the transmission cycle.

In the EU Member States, on the other hand, where the virus appears endemic in animal populations in many countries and has likely been so for a while (before the formal identification of the responsible agent), and where outbreaks of the disease were associated with a limited number of human cases and no fatalities, a systematic system of active surveillance on the whole territory could be considered expensive, difficult to implement, and with a limited impact on Public Health. As far as the Public Health risk is concerned, only focused measures of surveillance could be considered reasonable. WNV is already a subject of studies within the European Surveillance Network Group, the Task Force on Vaccines and Viral Diseases, and the European Network on Imported Viral Diseases and has been the subject of a joint meeting of these networks and national networks of the member states organised by DG SANCO in Luxembourg, 2003. Any organised plan of surveillance should be based on these networks and on the related multidisciplinary networks at the country level.

In the European context two strategies may be proposed according to the epidemiological context.

8.1. European countries free of outbreaks

A passive surveillance, based on differential diagnosis of clinically identified cases of encephalitis in humans and horses, limited to risk areas (migratory birds sanctuary, delta, etc), in a relevant epidemiological context, seems sufficient. It, thus, implies that a reference laboratory is designated, either at the national or at the European level.

8.2. European countries with reported outbreaks

It is likely that in countries with reported outbreaks, WNV circulates at low or undetectable level unless unknown factors lead to an outbreak. In this instance, any aetiological diagnosis of encephalitis in humans and horses may include WNV for its differential diagnosis.

When the structures make it possible, active surveillance in horses limited to the areas where WNV occurred as well as a surveillance based on sentinel birds (chickens and ducks) and passive surveillance of wild and domestic bird mortality can be performed. Any unusual die off in animals constitutes an alarm signal. A systematic serology according to a specific sampling plan may also be undertaken prospectively in selected places.

Cooperation with existing networks is necessary and could reduce associated costs while improving efficacy. These networks include those dedicated to neurological disorder (encephalitis) surveillance in humans and horses and on viral diseases in humans and animals. Close cooperation should be encouraged with research networks on floods and climate abnormalities in Europe that could predict at any time suitable conditions for the development of mosquito populations in expected as well as unexpected areas, and thus focus serological surveys to these areas before the emergence of cases.

9. CONCLUSIONS

WNV infection is an emerging zoonosis. Its future course is unpredictable and depends on 1) the distribution of reservoirs; 2) the distribution of vectors; 3) largely unknown factors (climate changes, virulence of isolates/strains of virus) that may modify intensity of infection and/or accessibility and susceptibility of the hosts.

WNV is a flavivirus with a complex life cycle involving a non-human primary vertebrate host (birds) and a primary vector (mosquito of the *Culex* genus). WNV is amplified during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and birds.

Humans, horses, and most other mammals are incidental or "dead-end" hosts because they do not produce significant viraemia, and do not contribute to the transmission cycle, except, for humans, through transplanted organs and blood transfusion as well as vertical, mother-to-child, transmission. The main route of human infection with WNV is through the bite of an infected mosquito.

WNV infection is usually asymptomatic in humans. The symptoms of severe infection are mostly related to West Nile encephalitis or meningitis; 0.6% of cases (mostly in childhood and elderly) will develop a severe form of disease, possibly with a fatal outcome.

No fatal case has been reported in EU Member States during outbreaks of WNV infection during the last decade (in Italy and France). However, during the past 8 years, 21 fatalities have been observed in Romania, 40 in Russia and 254 in the USA, all in urban outbreaks and in patients over 50 years of age.

Sensitive and specific methods of West Nile virus identification on (brain) biopsy materials from birds, horses and humans are available. The most efficient diagnostic method is detection of IgM antibody to WNV. The IgM MAC-ELISA is optimal for IgM detection because it is simple, sensitive and applicable to serum and Cerebro-Spinal Fluid samples as a screening test. Initial serologically positive samples should be confirmed by neutralisation test if collected in regions where different flaviviruses circulate.

In EU Member States Equine encephalomyelitis of all types (including WNV), are compulsory notifiable diseases, under control of the Official Veterinary Services.

Several retrospective and prospective serological surveys have been performed on horses in at risk areas. Passive surveillance of encephalitis/encephalo-meningitis cases in humans has been implemented in those countries where outbreaks had occurred within the past decade, and this surveillance has disclosed sporadic cases.

As far as surveillance in animals is concerned, horses appear to be good sentinels/indicators for the public health risk. In each European country, reference laboratories have the ability to achieve the biological diagnosis and/or quality control; horses are animals easy to sample; they are easily identifiable, either individually or by herds; they are involved in the same way as humans in the WNV epidemiological cycle, and serological surveys may detect other diseases of interest.

Birds could also be good sentinels/indicators; active surveillance of bird mortality is currently done in the USA. However, in Europe, bird mortality due to WNV is low, and the surveillance should rely on laboratory methods and would be more expensive with the necessity to dedicate specific personnel.

Mosquitoes do not appear to be good sentinels/indicators, mainly because of the lack of correlation between virus detection and epidemiological relevance.

10. RECOMMENDATIONS

In the European epidemiological context two strategies seem to be appropriate, depending on the previous occurrence of outbreaks.

1) All European countries should implement a passive surveillance strategy based on notification of clinically expressed encephalitis in humans and horses, followed by a documentation of the aetiological agent.

2) In European countries with reported WNV outbreaks, in addition, an active surveillance strategy should be implemented in horses, limited to those areas where

WNV outbreaks occurred. In addition a passive surveillance of wild and domestic bird mortality as well as surveillance based on sentinel domestic birds (chickens and ducks) could also be performed.

These strategies should be implemented through the surveillance networks on transmissible diseases already available at the European level, in cooperation with other networks involved in climate changes, flood, wetlands, bird repositories, all of which are risk factors linked to the vectors of WNV.

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