West Nile Virus Public Health Human Case Investigation Protocol

A. Case Definitions[1]

The information in the following table summarizes the key elements of the clinical and laboratory diagnostic criteria for the West Nile Virus (WNV) case classifications. More detailed information regarding the case definitions follows the table. Public health investigation is recommended for probable and confirmed cases only.

West Nile Virus Human Case Definitions: A Summary

Case Classification	Clinical Criteria	Suspect Case	Probable Case	Confirmed Case
West Nile virus Neurological Syndrome (WNNS)	History of exposure in an area with WNV activity **OR** History of exposure to an alternate mode of transmission **AND RECENT ONSET OF AT LEAST ONE OF:** • encephalitis • viral meningitis • acute flaccid paralysis • movement disorders • Parkinsonism or Parkinsonian-like conditions • other WNV-associated emerging clinical and neurological syndromes	Clinical criteria In the absence of or pending diagnostic test criteria, and in the absence of any other cause.	Clinical criteria **AND** At least one of the **Probable** diagnostic test criteria	Clinical Criteria **AND** At least one of the **Confirmed** diagnostic test criteria
West Nile virus Non-Neurological Syndrome (WN Non-NS)	History of exposure in an area with WNV activity **OR** History of exposure to an alternate mode of transmission **AND AT LEAST TWO OF:** • Fever • Myalgia • Arthralgia • Headache • Fatigue • Lymphadenopathy • Maculo-papular rash	Clinical criteria In the absence of or pending diagnostic test criteria, and in the absence of any other obvious cause.	Clinical criteria **AND** At least one of the **Probable** diagnostic test criteria	Clinical criteria **AND** At least one of the **Confirmed** diagnostic test criteria
West Nile virus Asymptomatic Infection	**Absence** of clinical criteria	Not applicable	Probable diagnostic test criteria **AND** Absence of clinical criteria	Confirmed diagnostic test criteria **AND** Absence of clinical criteria

Note: See West Nile virus Laboratory Diagnostic Test Criteria (p.5)

[1] The current case definitions have been adapted from the *National Surveillance Case Definitions* (updated May 11, 2006). They have been drafted with available information current at the time of writing. Case definitions and diagnostic test criteria are subject to change as new information becomes available.

1. West Nile Virus Neurological Syndrome (WNNS)

WNNS Clinical Criteria:

History of exposure in an area where WNV activity is occurring[2]

OR

History of exposure to an alternate mode of transmission[3]

AND AT LEAST ONE OF THE FOLLOWING:

- a neurological syndrome consistent with a diagnosis of encephalitis, or viral meningitis[4], or
- acute flaccid paralysis (poliomyelitis-like syndrome or Guillain-Barré-like syndrome)[5] or
- movement disorders (e.g. tremor, myoclonus), or
- Parkinsonism or Parkinsonian-like conditions (e.g. cogwheel rigidity, bradykinesia, or postural instability) or
- other WNV-associated emerging clinical and neurological syndromes[6].

Suspect Case:
Clinical criteria IN THE ABSENCE OF OR PENDING any diagnostic test criteria (see *Diagnostic Test Criteria* section) AND IN THE ABSENCE OF any other obvious cause.

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[2] History of exposure when and where WNV transmission is present, or could be present, or history of travel to an area with confirmed WNV activity in birds, horses, other mammals, sentinel chickens, mosquitoes, or humans.

[3] Alternate modes of transmission identified to date include: laboratory-acquired; *in utero*; receipt of blood components; organ/tissue transplant; possibly via breast milk and possible transmission of WNV to poultry workers in association with poultry outbreaks of WNV.

[4] Severe headache, high fever, stiff neck, alterations in the level of consciousness or mental status, any focal or generalized neurological disturbance such as cranial nerve deficits, paresis or paralysis, muscle weakness, sensory deficits, altered reflexes, involuntary movements or seizures.

[5] A significant feature of West Nile viral encephalitis may be marked muscle weakness, therefore WNV should be considered in the differential diagnosis of all suspected cases of acute flaccid paralysis that is more frequently unilateral, but could be bilateral, with or without sensory deficit. WNV-associated weakness typically affects one or more limbs (sometimes affecting one limb only). Muscle weakness may be the sole presenting feature of WNV illness (in the absence of other neurological features) or may develop in the setting of fever, altered reflexes, meningitis or encephalitis. Weakness typically develops early in the course of clinical infection. Patients should be carefully monitored for evolving weakness and in particular for acute neuromuscular respiratory failure, which is a severe manifestation associated with high morbidity and mortality. **For the purpose of WNNS classification, muscle weakness is characterized by severe (Polio-like), non-transient and prolonged symptoms. Electromyelography (EMG) and lumbar puncture should be performed to differentiate WNV-associated paralysis from acute demyelinating polyneuropathy (e.g. Guillain-Barre syndrome). Lymphocytic pleocytosis (an increase in WBC with a predominance of lymphocytes in the cerebrospinal fluid (CSF) is commonly seen in acute flaccid paralysis due to WNV whereas pleocytosis is not a feature of Guillain-Barre syndrome.** A person with WNV-associated acute flaccid paralysis may present with or without fever or mental status changes. Altered mental status could range from confusion to coma with or without additional signs of brain dysfunction (e.g. paresis or paralysis, cranial nerve palsies, sensory deficits, abnormal reflexes, generalised convulsions and abnormal movements).

[6] Rhabdomyolysis (acute destruction of skeletal muscle cells); peripheral neuropathy; polyradiculopathy; optic neuritis; and acute demyelinating encephalomyelitis. Other emerging clinical syndromes that have been reported include opthalalmologic conditions including chorioretinitis and vitritis. Facial weakness has been reported. Myocarditis, pancreatitis and fulminant hepatitis have not been identified in North America, but were reported in outbreaks of WNV in South Africa. **"Aseptic" meningitis without encephalitis or acute flaccid paralysis** occurring in August and September when WNV is circulating may be due to non-polio enteric viruses circulating at the same time. This should be considered in the differential diagnosis**. [Sejvar J et al. JAMA (2003) Vol. 290 (4) p. 511-515, Sejvar, J. et al. Emerg Infect Dis (2003) Vol9 (7) p. 788-93 and Burton, JM et al Can. J. Neruol. Sci. (2004) Vol.31 (2) p. 185-193]**

Probable Case:
Clinical criteria AND AT LEAST ONE of the probable case diagnostic test criteria (see *Diagnostic Test Criteria* section).

Confirmed Case:
Clinical criteria AND AT LEAST ONE of the confirmed case diagnostic test criteria (see *Diagnostic Test Criteria* section).[7]

Table 1. Diagnostic findings for West Nile virus neurological syndromes

Diagnosis	Clinical Presentation	CSF Findings	Neurological Imaging
West Nile menningitis	Signs of meningeal inflammation: nuchal rigidity; jolt accentuation of headache; Kernig or Brudzinski sign; or photophobia, without evidence of neuraxis involvement* Fever (≥38.0°C) or hypothermia (<35.0°)	Pleocytosis (≥5 leukocytes/mm^3)	Evidence of acute meningeal inflammation, however may present with normal findings. Neuroimaging is useful to rule out other pathology
West Nile encephalitis	Signs of encephalopathy: depressed or altered level of consciousness; lethargy; or personality change lasting >24 hours** Fever (≥38.0°C) or hypothermia (<35.0°); seizures, either new onset or exacerbationof previously controlled symptoms; focal neurological deficit; meningismus.	Pleocytosis (≥5 leukocytes/mm^3)	Consistent with acute inflammation (with or without meninges involvement) or acute demyelination; electroencephalograph findings consistent with encephalitis.
Acute flaccid paralysis	Acute onset of limb weakness with marked progression over 48-hour period*** Asymmetrical weakness; areflexia or affected limb(s); absence of pain, paresthesia or numbness in affected limb(s)	Pleocytosis (≥5 leukocytes/mm^3) and elevated protein levels (≥0.45g/dL.)	Electrodiagnostic studies (EMG, nerve conduction) consistent with an anterior horn cell process Spinal cord magnetic resonance imaging documenting abnormal increased signal in gray matter.

Source: Sejvar JJ, Haddad M. Tiernery B et al. JAMA 2003;290:511-5
*AND additional evidence of acute infection including 1 or more of the remaining findings (including CSF and neurological)
**AND additional evidence of central nervous system inflammation, including 2 or more remaining findings (including CSF and neurological)
***AND at least 2 of the remaining findings (including CSF and neurological)

[7] Note: A confirmatory PRNT or other kind of neutralization assay will be done only on a case by case basis in 2006, based on such factors as outlying exposure dates (i.e. cases that are identified earlier or later in the season that would be expected given historical and current season surveillance data); cases with a travel history, etc.

2. West Nile virus Non-Neurological Syndrome (WNV Non-NS)

Clinical Criteria:

History of exposure in an area where WNV activity is occurring[2] ;

OR

history of exposure to an alternate mode of transmission[3]

AND AT LEAST TWO of the following[8]:

- fever,
- muscle weakness, myalgia[9]
- arthralgia
- headache
- fatigue
- lymphadenopathy
- maculopapular rash

Suspect WN Non-NS Case:

Clinical criteria IN THE ABSENCE OF OR PENDING any diagnostic test criteria (see *Diagnostic Test Criteria* section) AND IN THE ABSENCE OF any other obvious cause.

Probable WN Non-NS Case:

Clinical criteria AND AT LEAST ONE of the probable case diagnostic test criteria (see *Diagnostic Test Criteria* section).

Confirmed WN Non-NS Case:

Clinical criteria AND AT LEAST ONE of the confirmed case diagnostic test criteria (see *Diagnostic Test Criteria* section).

[8] It is possible that other clinical symptoms could be identified that have not been listed and may accompany probable case or confirmed case diagnostic test criteria. For example, gastrointestinal (GI) symptoms were quite predominant in many case-patients in Canada and the USA in 2003 and 2004.

[9] Muscle weakness may be a presenting feature of WNV illness. For the purpose of WNV Non- Neurological Syndrome classification, muscle weakness or myalgia (muscle aches and pains) is characterized by a mild, transient, unlikely prolonged symptoms that are not associated with motor neuropathy.

3. West Nile virus Asymptomatic Infection (WNAI)[10]

Probable WNAI Case:

Probable case diagnostic test criteria (see *Diagnostic Test Criteria* section) IN THE ABSENCE of clinical criteria

Confirmed WNAI Case:

Confirmed case diagnostic test criteria (see *Diagnostic Test Criteria* section) IN THE ABSENCE of clinical criteria

B. West Nile virus Laboratory Diagnostic Test Criteria:

The information in the following table summarizes the key elements of the laboratory diagnostic test criteria for the WNV case classifications. More detailed information regarding the laboratory diagnostic test criteria follows the table.

WEST NILE VIRUS LABORATORY DIAGNOSTIC TEST CRITERIA: A Summary

AT LEAST ONE OF:	Probable Case Diagnostic Test Criteria	Confirmed Case Diagnostic Test Criteria
Plaque Reduction Neutralization Test (PRNT)		A 4-fold or greater change in WN virus neutralizing antibody titres (using a PRNT or other kind of neutralization assay) in paired acute and convalescent sera[11], or a single CSF specimen.
Flavivirus haemagglutination inhibition (HI)	A 4-fold or greater change in flavivirus HI titres in paired acute and convalescent sera, OR a titre of $\geq$ 1:320 in a single WN virus HI test.	Meets Probable Case diagnostic test criteria AND the detection of WN virus specific antibodies using a PRNT (acute or convalescent serum sample).[12]
West Nile Virus IgM ELISA	Detection of flavivirus antibodies in a single serum sample (or CSF if the CDC IgM ELISA is used) using a WN virus	Demonstration of flavivirus antibodies in a single serum sample using a WNV IgM ELISA, confirmed by the detection of WNV specific antibodies using a PRNT (acute or convalescent specimen).

[10] This category could include asymptomatic blood donors whose blood is screened using a Nucleic Acid Amplification Test (NAT), by Blood Operators (i.e. Canadian Blood Services or Hema-Quebec) and is subsequently brought to the attention of public health officials. The NAT assay that will be used by Blood Operators in Canada is designed to detect all viruses in the Japanese encephalitis (JE) serocomplex. The JE serocomplex includes WNV and 9 other viruses, although from this group only WNV and St Louis encephalitis virus are currently endemic to parts of North America. Further testing, outlined in part B, will be necessary to identify the specific virus from a blood donor with a reported positive donor screening test.

[11] Note: The recommended time frame between acute and convalescent sera is 10 to 14 days.(This applies to all references in the table where acute and convalescent sera required)

[12] Note: A confirmatory PRNT or other kind of neutralization assay will be required only on a case by case basis in 2006, based on such factors as outlying exposure dates (i.e. cases that are identified earlier or later in the season than would be expected given historical and current season surveillance data); cases with a travel history, etc.

AT LEAST ONE OF:	Probable Case Diagnostic Test Criteria	Confirmed Case Diagnostic Test Criteria
	IgM ELISA without confirmatory neutralization serology.	
WVN IgG ELISA[13]	An elevated titre in a WN virus IgG ELISA OR a significant increase in IgG antibody titres in paired acute and convalescent sera.	Demonstration of a seroconversion using a WNV IgG ELISA AND the detection of WNV specific antibodies using a PRNT (acute or convalescent serum sample).
Nucleic Acid Test (NAT) Screening test on donor blood (Canadian Blood Services)	Demonstration of Japanese Encephalitis (JE) serocomplex-specific genomic sequences	Confirmed by WNV-specific NAT.
Viral identification		Isolation of WNV from, or demonstration of WNV specific antigen or virus-specific genomic sequences in tissue, blood, CSF or other body fluids
IgG Avidity Testing: to distinguish between current cases and cases from previous season where titres are static in both acute and convaslescent paired sera	Presence of both IgM antibody and low avidity IgG in a convalescent serum sample	

Notes:

1. Public Health investigation is recommended for probable and confirmed cases only.

2. Immuno-compromised individuals may not be able to mount an immune response necessary for serological diagnosis. WNV diagnostic test criteria for these individuals should be discussed with Cadham Provincial Laboratory.

3. Longitudinal studies of encephalitis cases due to WNV have shown that WN virus-specific IgM antibody may persist in serum for 12 months or longer. Thus, the presence of serum anti-WN viral IgM antibody may not be diagnostic of *acute* WN viral infection in some cases, particularly in areas where WNV is known to have circulated previously. Seroconversion (by HI, IgG ELISA or PRNT assays) demonstrates a current WNV infection. Therefore, the collection of acute and convalescent sera for serologic analysis is particularly important to rule out diagnostic misinterpretation early in the WNV season (eg. May, June) and to identify initial cases in a specific jurisdiction. However it should be noted that seroconversions may not always be documented due to timing of acute sample collection (i.e. titres in acute sera may have already peaked). If static titres are

[13] Both CDC and commercial IgM/IgG ELISAs are now available for front line serological testing. Refer to appropriate assay procedures and kit inserts for the interpretations of test results.

observed in acute and convalescent paired sera, it is still possible the case may represent a recent infection. To help resolve this, IgG avidity testing[14] may be considered to distinguish between current and past infection. The presence of both IgM antibody and low avidity IgG in a patient's convalescent serum sample are consistent with current cases of viral associated illness. However test results that show the presence of IgM and high avidity IgG are indicative of exposures that have occurred in the previous season. Additional testing may be required in these cases, such as repeat serology or even CSF for IgM (if clinically indicated).[15]

4. PRNT and HT testing are not routinely performed in Manitoba. Only in special cases will other specimens be submitted for the PRNT and HT testing throughout the WNV season. Examples of these situations would be travel cases where the possibility of other viruses such as Dengue fever are being ruled out, cases occurring with outlying exposure dates (i.e. cases that are identified earlier or later in the season than would be expected given historical and current season surveillance data), or in the case of occurrence of death in WNV human case.

Probable Case Diagnostic Test Criteria:

AT LEAST ONE of the following are required as probable case diagnostic test criteria:

Detection of flavivirus antibodies in a single serum or CSF sample using a WN virus IgM ELISA without confirmatory neutralization serology (Eg. Plaque Reduction Neutralization Test –PRNT) **OR**
A 4-fold or greater change in flavivirus HI titres in paired acute and convalescent sera or demonstration of a seroconversion using a WNV IgG ELISA **OR**
A titre of $\geq$ 1:320 in a single WN virus HI test, or an elevated titre in a WNV IgG ELISA, **OR**
Demonstration of Japanese encephalitis (JE) serocomplex-specific genomic sequences in blood by NAT screening on donor blood, by Blood Operators in Canada..
Presence of both IgM antibody and low avidity IgG in a convalescent serum sample (Notes #3. in summary of West Nile Virus Laboratory Diagnostic Criteria – summary)

Note: Immunocompromised individuals may not be able to mount an immune response necessary for a serological diagnosis. West Nile virus diagnostic test criteria for these individuals should be discussed with a medical microbiologist.

[14] Early in infection the immune system generates antibodies that bind relatively weakly to viral antigen (low avidity). As the infection proceeds, an increasing percentage of newly generated IgG antibody displays higher binding affinity to virus antigen and thus avidity also rises (Note: avidity is usually measured based upon the ability of IgG to dissociate from antigen preparations after incubation with a solution of urea). As long as high avidity of IgG is not yet detected in the serum it can be assumed that the individual was exposed to the viral agent during a recent exposure. With respect to WNV infection it has not been precisely determined when (i.e. post-exposure) high avidity antibodies reach levels in serum that can be accurately detected by serological assays (there may be significant variation depending on the individual). However, it has been shown that greater than 95% of sera collected from individuals exposed to WNV 6-8 months previously will have IgG antibodies that bind strongly to viral antigen and will give high avidity scores using both IFA and ELISA testing formats. ***Note Avidity testing will not replace confirmatory neutralization testing, non- WNV flavivirus IgG antibody (e.g. dengue, SLE, etc.) may bind to the antigen preparations used in avidity assays. The Euroimmun West Nile virus IFA avidity test is now licenced for use in Canada by the Medical Devices Bureau (MDB). An ELISA format is currently being evaluated and is available from the company, however, it is not yet registered by MDB.***

[15] Roehrig et al., Emerging Infectious Diseases,Vol.9, No.3, March 2003

Confirmed Case Diagnostic Test Criteria:

The Confirmed Case Diagnostic Test Criteria will be used in situations where there is a positive travel history to areas endemic for other flaviviruses (eg: SLE, dengue), or where travel history is unknown. The Confirmed Case Diagnostic Test Criteria will also be used in cases occurring with outlying exposure dates (i.e. cases that are identified earlier or later in the season than would be expected given historical and current season surveillance data), or in the case of occurrence of death in a WNV human case. MOHs and physicians can request confirmatory tests if they have reason to believe that a probable case has an atypical presentation. Requests for confirmatory testing can be made to the National Microbiology Laboratory (NML) by Cadham Provincial Laboratory (CPL) or the Office of the CMOH.

AT LEAST ONE of the following are required as confirmed case diagnostic test criteria:

A 4-fold or greater change in WNV neutralizing antibody titres (using a PRNT or other kind of neutralization assay) in paired acute and convalescent sera, or CSF[16]. **OR**.
Isolation of WN virus from, or demonstration of WNV antigen or WNV-specific genomic sequences in tissue, blood, CSF or other body fluids or tissues such as brain **OR**
Demonstration of flavivirus antibodies in a single serum or CSF sample using a WN virus IgM ELISA, confirmed by the detection of WNV specific antibodies using a PRNT (acute or convalescent specimen). **OR**
A 4-fold or greater change in flavivirus HI titres in paired acute and convalescent sera or demonstration of a seroconversion using a WNV IgG ELISA **AND** the detection of WNV specific antibodies using a PRNT (acute or convalescent serum sample).

Notes:

1. Longitudinal studies of encephalitis cases due to WNV have shown that WNV-specific IgM antibody may persist in serum for 12 months or longer. Thus, the presence of serum anti-WN viral IgM antibody may not be diagnostic of *acute* WN viral infection in some cases, particularly in areas where WNV is known to have circulated previously. To resolve this issue, the use of of IgG avidity testing may be considered to distinguish between current and past infection (refer to Note in Probable Case Diagnostic Test Criteria. Additional testing may be required in these cases, such as repeat serology or even CSF for IgM (if clinically indicated).

2. Immunocompromised individuals may not be able to mount an immune response necessary for a serological diagnosis. WNV diagnostic test criteria for these individuals should be discussed with a medical microbiologist.

Clinical Presentation/Natural History

The majority of WNV infections are asymptomatic. It has been estimated that there are as many as 120 to 160 inapparent or mild infections for every severe clinical case. About 20% of infected persons develop the usually less severe symptom complex known as WNV non-Neurological

[16] Recommended time frame between acute and convalescent sera is 10 to 14 days.

Syndrome (WN Non-NS). This is usually a mild flu-like illness with fever, headache, and body aches, occasionally with skin rash and swollen lymph glands or other nonspecific symptoms that may typically last several days. Other signs and symptoms that may occur include nausea, vomiting, eye pain, or photophobia. Previously West Nile febrile illness or WN Non-NS was described as a mild acute syndrome lasting 3 to 6 days, but it can be a serious disease that takes several months to resolve (Drebot,M.A. and Artsob, H. "West Nile virus—Update for family physicians". Canadian Family Physician. 2005,51: 1094-1099).

WNV infection can cause severe, potentially fatal neurological illnesses. Symptoms of WNV meningitis, meningo-encephalitis or encephalitis may include headache, stiff neck, nausea, vomiting, and alterations in the level of consciousness (from mild lethargy to coma) or mental state (from mentally alert to confusion, delirium or disorientation). Virtually every possible type of neurological disturbance has been reported in viral encephalitis, including generalized or focal motor and sensory abnormalities, and seizures. Acute WNV infection has also been associated with acute flaccid paralysis, a polio-like syndrome which can present with acute onset of asymmetrical weakness without pain or sensory loss (*MMRW*, 51(37);825-828).

Some patients who have WNV encephalitis may experience serious sequelae, including permanent neurological deficits. At 12 months post-illness onset, 41-55% of patients affected in the 1999 New York outbreak self-reported that they had not recovered physically, functionally, or cognitively, and only 37% of patients reported full recovery in all three areas. The case fatality rate for persons with severe clinical illness is approximately 10% (range 4 to 14%), with higher ratios in older age groups and with virtually all deaths among encephalitis patients. (Campbell et. al, *The Lancet*, Vol.2, Sept. 2002, p.524).

For further advice on the clinical diagnosis of WNV, please see: Public Health Agency of Canada. *Management of Patients with West Nile Virus: Guidelines for Health Care Providers*. CCDR 2005:31S4:1-10.

Etiology

WNV is an arthropod-borne virus (arbovirus) of the genus flavivirus. WNV has been serologically classified with the Japanese Encephalitis Virus antigenic complex. The spherical virus particle consists of a host-derived lipid bilayer membrane containing a single positive-sense RNA genome of approximately 11,000 nucleotides. There are two distinct lineages of WNV strains. Those in lineage 1 have a worldwide distribution and have been associated with more significant human disease, whereas lineage 2 consists of enzootic strains from Africa. WN virus is indigenous to Africa, Asia, Europe and Australia, and was recently introduced to North America. Since 2002, there has been the emergence of a dominant North American Strain which occurs in 90% of isolates. This strain is very similar to the original strain introduced in 1999. Since 1999, very few genetic changes have occurred in the variant of WNV circulating in the United States (Petersen et al., *Annals of Internal Med*., Vol.137, No.3, 6 Aug. 2002. Davis et al. *Virology* 342: 252-265. Nov. 2005).

Epidemiology

Reservoir and Vectors:

- *Birds:* Birds are the main reservoir of WNV. The virus has been detected in over 300 species. Although birds infected with WNV can become ill and die, many species of infected birds survive. In North America, birds of the corvid family (including crows, ravens, blue jays, magpies and gray jays) are likely to die and therefore dead corvid surveillance is used in some jurisdictions as an indicator of virus presence.
- *Mosquitoes:* Mosquitoes are the main vectors of WNV. The primary mosquito vectors for WNV are in the *Culex* family. In North America, a number of *Culex* species have been implicated in transmission. *Culex tarsalis*, has been identified in Manitoba as the mosquito most likely to be responsible for human infections. *Culex tarsalis* will bite both birds and mammals. Mosquito surveillance is used to assist in the prediction of risk of human illness in most jurisdictions.
- *Pets and Livestock:* WNV can affect horses and has caused illness and death in horses. The use of smudges and sprays, and keeping horses indoors at night will help to reduce mosquito exposure. A West Nile virus vaccine for horses is available in Canada. Illness due to WNV has not been found among pigs and cattle. Illness in dogs and cats occurs very rarely.

Transmission:

WNV is amplified during periods of adult mosquito blood-feeding by continuous transmission between mosquito vectors and avian reservoir hosts. Infectious mosquitoes carry virus particles in the salivary glands and infect susceptible bird species during blood meal acquisition. Competent bird reservoirs develop a sufficient viremia to infect mosquitoes during biting. People, horses and most other mammals rarely develop a level of viremia sufficient for the continuation of a transmission cycle. They are, therefore, thought to be incidental or "dead-end" hosts.(Recent evidence suggests that animals may be involved in amplifying the virus in mosquito populations when many mosquitoes are feeding at once. Infected mosquitoes feeding on anesthetized mice led to the subsequent infection (within 1 hour) of uninfected mosquitoes feeding on the mice. (Higgs, S et al. PNAS (2005) Vol.102 No. 25 p. 8871-8874)..

In 2002, the US Center for Disease Control reported several other potential modes of transmission for WNV. Two cases were reported of WNV infection in laboratory workers, with no other known risk factors, who acquired the illness through **percutaneous inoculation** while handling tissues from infected birds in the lab (*MMWR* 2002,51(50); 1133-1135). There were also at least 47 cases in the USA of confirmed WNV infection after receiving **blood products** subsequently found to have evidence of WNV (*MMWR* 2002; 51: 833-6), or **transplantation of infected organ tissue (***MMWR* 2002, 51(39); 884, 895) .

Transplacental transmission from a previously healthy woman to her child occurred when she became ill with WN Non-NS in her 27th week of gestation. The child was born with laboratory evidence of congenitally acquired WNV infection (WNV specific IgM in blood samples), and was found to have ophthalmic and cerebral abnormalities (*MMWR* 2004, 53(07), 154-157). Although this case demonstrated intrauterine WNV infection in an infant with congenital abnormalities, it did not prove a causal relation between WNV infection and these abnormalities. During 2002, CDC Atlanta investigated three other instances of maternal WNV infection; all three infants were born at full term apparently normal and with negative laboratory tests for

WNV infection. A woman in Michigan was found to have WNV after receiving a post-partum blood transfusion, and her infant was subsequently found to be WNV positive after exposure to her **breast milk** (*MMWR* 2002 51(39); 877-878). CDC Atlanta is reviewing data on pregnancy outcomes for approximately 70 women with WNV illness during pregnancy in 2003 (*MMWR* 2004, 53(07), 154-157).

There is also some evidence that poultry workers exposed to a WNV outbreak among turkeys may have become infected with WNV by this exposure. (*MMWR 2003, 52(42)).*

Surveillance:

In Manitoba, a surveillance program is in place to detect WNV infection in *Culex tarsalis* mosquito larvae, mosquitoes, horses and humans. For more information on the Manitoba WNV surveillance program, please refer to the Manitoba Health web site at http://www.gov.mb.ca/health/wnv.

Occurrence:

WNV was first isolated from a febrile woman in the West Nile district of Uganda in 1937. Outbreaks of WNV have occurred in Africa, Egypt, Israel, Asia, Romania, Russia and France. Before 1999, however, WNV had never been found in the Americas. A possible explanation for its appearance on this continent is that the virus was introduced by an infected bird or mosquito that was imported or, less likely, an infected person returning from a country where the virus is common. In 1999, 62 cases of severe disease, including 7 deaths, occurred in the New York area. In 2000, 21 cases were reported in the USA, including 2 deaths in the New York City area. In 2001, there were 66 human cases of severe disease and 9 deaths in the USA. In 2002, the American numbers had increased to 4156 cases, and 284 deaths. By 2003, there were 9862 cases of WNV and 264 deaths in the USA. In 2004, there were 2,470 cases of WNV in the USA which occurred in individuals ranging from one month to 99 years of age, 900 of which were reported as West Nile meningitis or encephalitis. This resulted in 88 deaths for 2004.

The first reported case of locally acquired disease due to WNV in Canada was reported in Ontario in September of 2002. The total numbers of cases from 2002 were as follows: Quebec reported 19 confirmed cases, two deaths and no probable cases; Ontario reported 319 confirmed and 86 probable cases, and 18 deaths. Two confirmed travel-related cases were identified in Alberta. Other surveillance activities identified WNV activity in five provinces – Nova Scotia, Quebec, Ontario, Manitoba and Saskatchewan.

In 2003, a total of 1388 cases of WNV and 14 deaths were reported in Canada, with the majority occurring in Saskatchewan (848 cases). In 2003 and 2004, locally acquired human cases of WNV were identified in Alberta, Saskatchewan, Manitoba, Ontario and Quebec. The total number of human cases in Canada for 2004 was 25 cases. In 2005, there was a total of 236 cases of WNV reported in Canada.

In Manitoba during the summer of 2002, WNV was detected in birds, sentinel chickens, horses and mosquitoes. In 2003, the first human cases were identified. There were 143 human cases of WNV infection identified, 35 of whom were diagnosed with WNNS. There were 2 deaths related to WNV in 2003 in Manitoba. In 2004, there were 3 human cases of WNV infection identified, one of which was diagnosed with WNNS. There were no deaths related to WNV in 2004 in

Manitoba. In 2005, there were 58 human cases of WNV infection, of which 10 were diagnosed as WNNS. There was one death in an individual who had a WNV infection.

For updated information describing current WNV activity in Manitoba, Canada and the United States, please refer to the Manitoba Health web site at http://www.gov.mb.ca/health/wnv the Public Health Agency of Canada website at http://www.phac-aspc.gc.ca/wnv-vwn and the CDC website at http://www.cdc.gov/ncidod/dvbid/westnile/surv&control.htm.

Incubation Period:

In cases of clinical illness, the incubation period ranges from 2 to 15 days. However, prolonged periods of up to 21 days have been observed in patients following organ transplantation. The period of viremia begins several days (up to 6-7 days) before the onset of clinical illness and ends shortly after symptom onset. (Public Health Agency of Canada Management of Patients with West Nile Virus: Guidelines for Health Care Providers. CCDR 2005:31S4: p.2.).

Susceptibility and Resistance:

Susceptibility may be universal. In the United States infection has occurred in individuals 9 months to 94 years of age. It is likely persons infected with WNV are immune to subsequent infections with this virus, however longitudinal studies have not been completed to confirm this. Studies in Egypt in the 1950's, in an area where WNV circulates most years; and where uncomplicated WN Non-NS is a mild and common childhood disease, high prevalence of antibodies was observed which increased with age. WN Non-NS epidemics and WN encephalitis cases were rare (Taylor et. al., *Am J Trop Med Hyg*, 1956; 5: 579-620).

Illness has been more frequent and more severe in persons over the age of 50, individuals with weakened immune systems (immunocompromised) and organ transplant recipients and in individuals with other chronic diseases. In any population affected by WNV, the proportions of different clinical syndromes will depend on the previous history of WN viral activity in the area and the consequent level of background immunity in the population, the age structure of the population and the focus and completeness of surveillance and public education efforts (Campbell et al., *The Lancet*, Vol.2, Sept. 2002; 521)

Period of communicability:

The risk of WNV varies from year to year. The risk is influenced by precipitation, temperature, mosquito populations and many other factors. Manitobans are at highest risk of being bitten by a WNV-infected mosquito in July, August and September. In 2003, the first known human case was infected in July and the last case of the season was exposed in early September.

WNV has been transmitted directly from person to person by blood transfusion or tissue/organ donation. WNV infection has also been demonstrated in utero and can be transmitted through breast feeding. In otherwise healthy infected persons, virus can generally be isolated from blood during peak viremia that occurs from 2 days before until about 4 days after illness onset, but the success of virus isolation sharply decreases after the first day of illness (Campbell et al.)

Laboratory Diagnosis

In Manitoba, WNV is transmitted to humans primarily by the *Culex tarsalis* mosquito, usually during July, August, and early September. WNV should also be considered in patients with a travel history to areas where the virus is circulating or who have other risk factors for WNV, such as being a transplant recipient.

Patients with symptoms and/or signs consistent with encephalitis, meningitis, acute flaccid paralysis, or other WNV-associated emerging clinical syndromes should be tested for WNV. These symptoms should be stated on the Cadham Provincial Laboratory (CPL) requisition to assure priority testing. Routine testing of patients with mild illness is not expected. Samples should be submitted when the result will assist in the management of the patient.

The specimen of choice for WNV testing is blood serum. Serologic testing is the most sensitive test for WNV. The initial test that is carried out is the IgM antibody Elisa test. Both acute and convalescent samples may be required as IgM antibodies may not be detectable within the first seven days of symptoms. Unless contraindicated, an LP should be performed to obtain CSF for cases suspect of neurologic disease. CSF findings are valuable in the classification of cases.

To avoid delays in test processing, all of the following information should be identified on the CPL requisition:

- Patient and physician information;
- Clinical information (meningitis, encephalitis, acute flaccid paralysis or other WNV-associated emerging clinical syndrome) and all CSF test results;
- Travel history within the last month
- History of immunization against Japanese encephalitis and Yellow Fever.

- **Blood (5 to 10 cc in adults, 2 to 3 cc in children)** is the specimen of choice for detection of WNV antibodies and should be collected and submitted to CPL for every case of suspected WNNS. Obtain convalescent sera in the same fashion 10-14 days later after collection of the acute specimen in patients who do not have an alternate diagnosis (e.g. enteroviral meningitis). Paired sera are usually necessary to confirm the diagnosis of acute WNV infection.

If a CSF specimen has been submitted, and pleocytosis (>5 white blood cells /mm^3) or elevated protein are identified, standard diagnostic tests, including tests to rule out bacterial infection should be undertaken, as usual.

- Cerebrospinal fluid, 2 to 3 cc in a sterile container, should be collected and submitted to Cadham Provincial Laboratory (CPL) for viral studies. Testing will be conducted for common causes of viral meningitis (e.g. enterovirus).

No specific treatment for WNV infection exists, and the consequences of WNV infection during pregnancy have not been well defined. For these reasons, screening of asymptomatic pregnant women for WNV infection is not recommended (*MMWR* 2004, 53(07), 154-157). Pregnant women who have encephalitis, meningitis, acute flaccid paralysis or other evidence of WNV-associated emerging clinical syndromes should have serum (and CSF if clinically indicated) tested for WNV as above.(MMWR 2004, 53(07). 154-157. MMWR:2004 51, 1135-1136, CDC2002: “Intrauterine West Nile virus infection” and Alpert,S.G. Ferguson,I and Noel,L.P.

"Intrauterine West Nile Virus: Ocular and systemic findings. American Journal of Opthalmology, 136, 733-735.)

Public Health Investigation

Public Health investigation is required for probable and confirmed cases only. ***Samples from patients with milder illness as seen with WNV Non-NS should be submitted only when the test result will assist with the management of the patient. While all of the following sections are included in the human case investigation report form, the information below may provide further useful explanatory detail regarding key elements of the public health investigation.***

Organ and/or blood/blood-products donation/receipt

- Has the person donated/received organs, blood or blood products within the 2nd to 15th day prior to the onset of symptoms or the date of a positive blood donor screen test (yes, no, unknown). If yes, specify date(s), and indicate whether the donor organization has been notified.

Medical Conditions

- Any significant underlying chronic medical conditions? If yes, specify
- The evaluation of pregnant women and infants. See CDC.gov/ncidod/dvbid/qu/breastfeeding.html (CDC. (2004). for Interim guidelines for the evaluation of infants born to mothers infected with WNV during pregnancy. MMWR, 53, 154-157).
- If patient is pregnant or breast feeding, advise physician to consult an infectious disease specialist.

Travel History

- Travel outside of city or municipality of residence between the 2nd to 15th day prior to the onset of symptoms or date of a positive blood donor screen test? (yes, no, unknown)
- If yes, specify. (During the 2nd to 15th day prior to the onset of symptoms or date of a positive blood donor screen test, have you traveled within or outside of the province/country? Location, duration, dates)
- History of travel-related illness

Immunization and Flavivirus Infection history

- Has the person received Japanese Encephalitis (JE) vaccine in the past? (yes, no, refused, don't know/unsure) If yes, specify dates.
- Has the person received Yellow Fever vaccine in the past? (yes, no, refused, don't know/unsure). If yes, specify dates.
- Is there a history of vaccination against other arboviruses? (yes, no, refused, don't know/unsure).
- History of WNV, Western Equine Encephalitis infection (WEE), Dengue fever, St. Louis Encephalitis (SLE), Yellow Fever, Powassan infection If yes, specify date.

Exposures/Risk Factors

- Use of insect repellant(s) when outdoors (never, sometimes, most of the time, always)
- Use of insect repellant that contains DEET? (yes, no, unsure/don't know)

- Outdoor occupation or recreational activities during the evening, at night and early morning when *Culex tarsalis* is most active.
- History of exposure to mosquito bites within the 2nd to 15th day prior to the onset of symptoms or date of a positive blood donor screen test, particularly if between dusk and dawn. If yes, specify date, time of day, place.
- History of handling potentially infected birds/animals within the 2nd to 15th day prior to the onset of symptoms or date of a positive blood donor screen test? If yes, specify date and location.
- History of working with potentially contaminated tissue.
- Most likely location of exposure is determined by looking back at the potential locations where the person may have been exposed to mosquito bites within the 2nd to 15th day prior to the onset of symptoms. This information is asked in order to assist in WNV program planning.

Control

Management of Cases:

- Investigation for location and circumstances of exposure and infection.

Treatment:

- Supportive
- No antiviral treatment is considered of benefit at this time.

Management of Contacts:

- Contact tracing is not required if mosquitoes are thought to be the most likely mechanism of exposure to WNV. If mosquitoes are not thought to be the most likely mechanism of exposure to WNV, the OCMOH should be notified and will determine if further investigation is required.
- The CDC Unit and Cadham Provincial Laboratory will notify Canadian Blood Services with regard to potential blood product related exposure.
- No human vaccine currently exists for WNV.

Reporting Requirements

Report the following to the Director of Communicable Disease Control, Manitoba Health, by telephone (204-788-6736) or secure fax (204-948-3044):

- Encephalitis in all ages of any cause remains a reportable disease in Manitoba.
- All positive laboratory results indicating the presence of WNV infection are reportable by laboratories and Canadian Blood Services to Communicable Disease Control, Manitoba Health.
- Health professionals and laboratories should also report observed outbreaks or unusual clusters of symptoms, signs or lab results of any communicable disease.

Specific reporting processes are as follows:

1. CDC Unit, Manitoba Health reporting to regions:

The CDC Unit will, upon receipt, refer positive laboratory reports of probable or confirmed cases to regions. When forwarding these referrals, the CDC Unit will specify whether the classification is a "confirmed case".

2. Reporting by Regional Public Health to the CDC Unit:

Public Health investigation and reporting is required for probable and confirmed cases only. During the summer season when peak exposure and reporting of human cases is expected (mid-July to mid-September), public health reporting may occur in a two-step process that will accommodate immediate needs for necessary information to inform communications, program and mosquito control decision making, and to allow reasonable time frames for regional public health to complete the investigation and submit the completed form to the CDC Unit:

Step 1: Regional public health practitioners will have(five working days)from the receipt of the WN virus positive laboratory referral, to forward key data elements as highlighted on the investigation form, to the CDC Unit. The CDC Unit will enter this core information into the provincial surveillance database within 24 hours of receiving it from the region.

Step 2: Regional public health practitioners will forward completed investigation forms to the CDC Unit, within 30 working days of having received the positive laboratory report.

3. Reporting by Manitoba Health to Canadian Blood Services (CBS):

Manitoba Health will forward to CBS, immediately upon receipt, reports of probable and confirmed cases of WN virus infection. This notification will occur by fax from the CDC Unit. Cadham Provincial Laboratory staff will fax positive confirmed human case reports to the Medical Director, CBS Manitoba, rather than hand delivering positive results as in previous years:

4. Reporting by CBS to Manitoba Health:

CBS will report to Director of Communicable Disease Control or designate, any positive laboratory results arising from screening tests done on donor blood.
a) For the first case each season, CBS will page Manitoba Health with the positive laboratory result.
b) For all subsequent positive lab reports, CBS will notify Manitoba Health the next business day, by telephone (788-6736) or secure fax (948-3044) of the following information: Name, Date of Birth, Address, Specimen Date, Test Result.

Preventive Measures

WNV and other arboviral encephalitis can be prevented in two major ways: (a) personal protective measures to reduce contact with mosquitoes; and, (b) measures to reduce the population of infected mosquitoes in the environment.

1. *Educate the public about WNV transmission by mosquitoes and personal protection, including the following:*

    - If outside between dusk and dawn when mosquitoes are most active, or during the day in an area where there are weeds, tall grass, or bushes, people should consider the use of an appropriate insect repellent, and should wear loose-fitting, light-coloured protective clothing, such as long pants, loose-fitting, long-sleeved shirts, and socks,. For more information on the use of mosquito repellents, please refer to the Health Canada's Pest Management Regulatory Agency website at: *http://www.pmra-arla.gc.ca/english/consum/mosquito-e.html*, or, the Manitoba Health fact sheet at *http://gov.mb.ca/health/wnv*.

    - Mosquitoes can enter homes through unscreened windows or doors, or broken screens. Make sure that doors and windows have tight-fitting screens. Repair or replace all screens that have tears or holes.

2. *Educate the public about source water reduction methods to reduce the population of infected mosquitoes in the environment. General principles include:*

    - Eliminate unnecessary standing water that collects on your property.

For more specific information on ways to reduce mosquito populations, refer to the Information Sheet entitled *"Effective Control of Mosquitoes Around Your Home"* on Health Canada's Pest Management Regulatory Agency website at *http://www.pmra-arla.gc.ca/english/consum/mosquito-e.html* or the Manitoba Health fact sheet at *http://gov.mb.ca/health/wnv*.

3. *Mosquito control activities may also be conducted at the municipal and/or provincial level.*

    Based upon an assessment of human risk and the appropriateness and feasibility of mosquito control measures, including reduction of standing water sites, larviciding or mosquito adulticiding, may be considered in order to reduce mosquito populations. The considerations involved in mosquito control are outlined in the document *"West Nile Virus Program 2006: Planning Document for Municipalities – April, 2006" available on the Manitoba Health website http://gov.mb.ca/health/wnv*.