

# Diagnosis

## Clinical signs

West Nile virus causes disease in humans, horses, and several species of birds. Most infected individuals show few signs of illness, but some develop severe neurological illness which can be fatal.

### Birds

Most species of birds can become infected with WNV. Incubation period usually is 3-4 days and the clinical outcome of infection is variable. Some species appear resistant while others suffer fatal neurologic disease. Commonly symptoms observed are:

- depression,
- lethargy,
- ruffled feathers,
- weight loss ataxia,
- paralysis,
- handling movements,
- pedaling,
- stiff neck,

- opisthotonos,
- motor incoordination.

Death usually occurs 24 hours after the onset of nervous symptoms.

## Horses

The incubation period for equine WN encephalitis following mosquito transmission is estimated to be 3–15 days. A fleeting viraemia of low virus titre precedes clinical onset ([Bunning et al., 2002](#)). WN viral encephalitis occurs in only a small per cent of infected horses; the majority of infected horses do not display clinical signs ([Ostlund et al., 2000](#)). The disease in horses is frequently characterised by mild to severe ataxia. Additionally, horses may exhibit weakness, muscle fasciculation and cranial nerve deficits ([Cantile et al., 2000](#); [Ostlund et al., 2000](#); [Snook et al., 2001](#)). Fever is an inconsistently recognised feature. The clinical signs can resolve with healing in 5–15 days or progress rapidly with death of the subjects. Treatment is supportive and signs may resolve or progress to terminal recumbency. The mortality rate is approximately one in three clinically affected unvaccinated horses.

## Humans

Most people infected with West Nile virus do not develop any symptoms. The incubation period for clinical illness generally ranges from 2 to 14 days, but prolonged incubation periods of up to 21 days have been observed among immunocompromised patients. West Nile fever can range from a mild infirmity lasting a few days to a debilitating illness lasting weeks to months. Symptoms are of sudden onset and often include headache, malaise, fever, myalgia, chills, vomiting, rash, fatigue, and eye pain ([Zhou et al., 2010](#)). Less than 1% of people infected via mosquito bite develop West Nile Neuroinvasive Disease Meningitis characterized by clinical signs of meningeal inflammation, including nuchal rigidity or photophobia associated with encephalitis characterized by depressed or altered level of consciousness, lethargy or acute flaccid paralysis ([Sejvar et al., 2008](#)). All ages are affected, although very strong predilection is shown with advancing age. Case fatality rates among patients with neuroinvasive disease generally approximate 10%. Advanced age is the most important risk factor for death, ranging from 0.8% to 17% in those aged at least 70 years ([Lindsey et al., 2010](#)). Treatment is supportive

and illness duration varies from weeks to months with possible long-term functional and cognitive difficulties.

## Anatomo pathological lesions

### Birds

In birds the most important lesions are characterized by:

- meningoencephalitis with a marked involvement of the Purkinje cells of the cerebellum, [\(Monaco et al, 2015\)](#)
- splenomegaly,
- myocarditis,
- liver and kidney involvement.

### Horses

There are no macroscopic lesions affecting the organs, the lesions are visible only at the microscopic level and are exclusively affecting the central nervous system.

### Humans

Anatomo-pathological lesions in humans are limited to the presence of necrotic foci with infiltration of polymorphonuclear leukocytes and macrophages affecting the central nervous system, liver and heart.

## Laboratory diagnosis

Due to the occurrence of inapparent WNV infections, diagnostic criteria must include a combination of clinical evaluation and laboratory tests. Moreover, diagnostic tests are

influenced by the great variability of the West Nile virus genome, the cross-reactivity with other *Flaviviruses*, the transient viraemia and the low viral load developed during the infection. These elements hamper the diagnostic pathway, which must be performed by specialized laboratories and reference centers.

In Italy, all the samples collected within of veterinary surveillance are tested by the network of the Istituti Zooprofilattici Sperimentali (IIZZSS) located throughout Italy and positive samples have to be confirmed by the National Reference Center for the Foreign Diseases of Animals (CESME) held by the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise (IZSAM).

The available and most used tests for the direct and indirect diagnosis of WNV infection are:

#### **Virological diagnosis:**

- Molecular biology techniques (RT-PCR and real time PCR),
- Viral isolation,
- Immunofluorescence,
- Immunohistochemistry.

#### **Serological diagnosis**

- IgM ELISA;
- IgG ELISA;
- Microtitre seroneutralization;
- Plaque reduction neutralization test.

In the last years, the enzyme immunoassays (ELISA) have been widely used due of their great specificity and sensitivity, as well as rapid execution. However, cross-reactivity between *Flaviviruses* belonging to the same serocomplex, as for West Nile and Usutu viruses, requires to confirm ELISA positive results by using the seroneutralization test. Within the national veterinary surveillance activities the diagnostic to define a suspected and confirmed case of WNV infection are defined in «**Integrated national plan for prevention, surveillance and response to West Nile and Usutu viruses - 2019**».

