



Prepared in cooperation with the Canadian Wildlife Health Cooperative

West Nile Virus (Avian) Case Definition for Wildlife

Chapter 1 of Section C, Virus

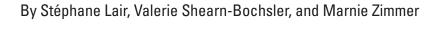
Book 19, Wildlife Disease Case Definitions



Techniques and Methods 19-C1



West Nile Virus (Avian) Case Definition for Wildlife





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IHC immunohistochemistry

PCR polymerase chain reaction

WNV West Nile virus

West Nile Virus (Avian) Case Definition for Wildlife

By Stéphane Lair, 1 Valerie Shearn-Bochsler, 2 and Marnie Zimmer 1

Introduction

Diagnostic laboratories receive carcasses and samples for diagnostic evaluation and pathogen/toxin detection. The intent of a case definition is to provide scientifically based criteria for determining (1) if an individual carcass has a specific disease and the confidence of that diagnosis; and (2) if a pathogen or toxin is evident in a carcass or sample (for example, swab, tissue sample, skin scraping, blood/serum sample, environmental sample, or other). Using these criteria, cases diagnosed with a specific disease (diagnosing disease) will be classified as "confirmed," "presumptive," or "suspected;" and evidence of a pathogen or toxin (detecting pathogen/toxin) will be classified as "exposed" or "present/detected." Classification is based on a combination of factors: individual, place, time, history, clinical signs, diagnostic observations, and (or) diagnostic test results. Case definitions can bring clarity and consistency to the evaluation process. Their use within and between organizations allows more uniform reporting of diseases and etio-

Case definitions are proposed for use in wildlife diagnostic laboratories and are not intended to replace regulatory standards provided by Government reporting agencies. Ideally, case definitions would be updated periodically as new information becomes available and new test methods are developed. Refer to the glossary for terminology definitions.

Disease/condition.—West Nile virus (WNV), West Nile infection

Pathogen/toxin etiologic agent.—West Nile virus (*Orthoflavivirus* sp.) The genus name was changed from *Flavivirus* to *Orthoflavivirus* (Walker and others, 2020).

Scope of the Case Definition

This case definition applies to avian species.

Case Definition Criteria

The case definition criteria are a concise summary of the current science regarding the clinical signs, history, gross and microscopic observations, and laboratory test results associated with a specific disease or pathogen. Various combinations of the criteria result in different case classifications representing the degree of certainty of the diagnosis.

Individual, Place, and Time Criteria for Diagnosis and Testing

Individual.—WNV has been documented in over 300 species of birds and mammals (Ip and others, 2014). Corvidae ssp. (Leach, 1820) (Corvids), *Pelecanus* ssp. (Linnaeus, 1758) (pelicans), and Falconiformes ssp. (raptors) are particularly susceptible; however, in 2013, Utah, an outbreak in *Podiceps nigricollis* (C. L. Brehm, 1831) (eared grebes) killed thousands of these birds (Ellis and others, 2007; Ip and others, 2014; Centers for Disease Control and Prevention, 2016).

Place.—Broad geographic range in North America, and most Central and South American countries (Diaz and others, 2008; McLean 2006).

Time.—Generally during mosquito season although has occurred in winter. In North America, mosquito season is in April to November depending on the State or Province; cases peak in August and September (McLean 2006; Ip and others, 2014).

Field Criteria for Diagnosis

History and clinical signs.—Diagnostically compatible illness may present with lethargy, ataxia, tremor, blindness, unusual posture, inability to perch or stand, lack of awareness surrounding, recumbency, seizures, and death. Abnormal molting and persistently abnormal feathers have been described in some species of raptors (Ip and others, 2014; Wunschmann and others, 2014).

Other.—Severity of clinical signs can vary by species (Ellis and others, 2007; Ip and others, 2014). Because of the immunomodulatory effect of the virus, opportunistic infections, such as acute aspergillosis or septicemia, are common concomitant findings, especially in diurnal raptors.

¹Canadian Wildlife Health Cooperative.

²U.S. Geological Survey.

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Laboratory Criteria for Diagnosis

Gross examination.—Diagnostically compatible findings in acute deaths (mostly in corvids) may include birds that may be well nourished with no gross lesions. In birds with neurological signs (especially raptors), diagnostically compatible postmortem findings may include emaciation with or without areas of pallor in the heart and pectoral muscles. Hepatomegaly and splenomegaly could be present, especially in strigiform. In nestling and fledgling birds, especially pelicans, emaciation, myocardial pallor, and splenomegaly may be present. Gross lesions may vary by species (Ellis and others, 2007; Wunschmann and others, 2014).

Histopathology.—Histopathological findings may include multifocal to coalescing mononuclear necrotizing myocarditis and myositis with or without fibrosis, and non-suppurative encephalitis or meningoencephalitis characterized by gliosis and perivascular lymphoplasmacytic cuffing (especially in diurnal raptors). Other histological lesions include endophthalmitis, hepatitis, lymphoid depletion of the spleen and the bursa, splenic and hepatic hemosiderosis, pancreatitis, cryptitis, and ganglioneuritis. Multifocal to coalescing splenic necrosis is often observed in owls. Lesions are often very subtle in corvids in that they can be limited to mild multifocal intestinal necrotizing cryptitis. Histopathological lesions may vary by species (Ellis and others, 2007; Wunschmann and others, 2014).

Diagnostic test(s).—Virus isolation, polymerase chain reaction (PCR), immunohistochemistry (IHC), or serology (Lanciotti and others, 2000; Ellis and others, 2007; Wunschmann and others, 2014).

Laboratory Criteria Categorization

Laboratory confirmed.—Corvids: Positive PCR, IHC, or virus isolation in Vero cells from feather pulp, kidney/spleen pool, brain, or liver (positive cultures are confirmed as WNV via PCR), with or without gross and histopathological lesions. Note that, for virus isolation, brain is the preferred tissue for cultures in birds presenting with emaciation or neurological signs. In the absence of histopathological lesions, no other cause of mortality is identified.

Raptors and other species: Consistent gross and (or) histopathological lesions and positive PCR or IHC result or positive virus isolation in Vero cells from feather pulp, kidney/spleen pool, brain, or liver (positive cultures are confirmed as WNV via PCR). Note that, for virus isolation, brain is the preferred tissue for cultures in birds presenting with emaciation or neurological signs.

Laboratory supportive.—Corvids: Characteristic gross and histopathological lesions without a positive PCR or IHC, or viral culture result (either the testing was not done or the results are negative or inconclusive).

Raptors and other species: Characteristic gross and histopathological lesions without a positive PCR or IHC, or viral culture result (either the testing was not done or the

results are negative or inconclusive); or positive virus isolation (confirmed by PCR) or PCR or IHC result but no tissues available for examination or no consistent histopathological lesions.

Exposed.—Serology positive in the absence of any evidence of overt signs of disease. Positive serology in the U.S. Geological Survey National Wildlife Health Center Diagnostic Virology Laboratory was reactivity greater than 1:40 (Plaque Reduction Neutralization Test assay). Titer must be fourfold greater for WNV than the St. Louis Encephalitis virus.

Present/detected.—Positive PCR or virus isolation in the absence of gross or histological lesions of WNV.

Supplemental Diagnostic Information

Additional diagnostic comments.—Because neutralizing antibodies to the St. Louis encephalitis virus can cross react with WNV and the St. Louis encephalitis virus circulates in birds in the same geographic areas, serum should be tested against WNV and St. Louis encephalitis virus. Be aware that some birds in managed conservation programs such as Grus americana (Linnaeus, 1758) (whooping cranes), Gymnogyps californianus (Shaw, 1797) (California condors), and Centrocercus urophasianus (Bonaparte, 1827) (greater sage grouse) may have been vaccinated against WNV, which may result in positive serology findings. Review of vaccination status is warranted.

Notifiable/reportable disease.—This is considered a reportable disease at the State, Provincial, and (or) Federal level in Canada and the United States. Please check and report to the appropriate agricultural and (or) public health authorities as needed.

Epidemiologic Linkage Criteria for Diagnosis

An epidemiologic linkage can be established by close geographic and temporal proximity (in other words, part of the same mortality event) as one or more confirmed cases of WNV or at a site with a recent history of confirmed WNV with similar presentation as described in the "Case Definition Criteria" section.

Case Classification

The sum of the criteria listed in the "Case Definition Criteria" section (individual, place, time, field, laboratory, and epidemiologic linkage criteria) associated with a particular disease or pathogen/toxin in an individual animal or specimen add up to a case classification (fig. 1; table 1).

Depending on the confidence in the results, cases of a specific disease will be classified as "confirmed," "presumptive," or "suspected;" and evidence of a pathogen or toxin will be classified as "exposed" or "present/detected" (table 1; refer

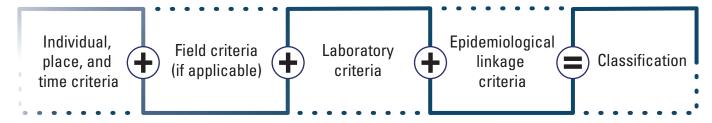


Figure 1. Case definition criteria add up to the case classifications. From Miller and others (2024).

to glossary for definitions). A specific case classification may have more than one pathway to it. Not all classifications may be used for every disease. Compatible epidemiological linkage criteria are required for the "suspected" case classification.

Note.—The field and laboratory criteria in table 1 reflect the typical presentation of WNV. The exact presentation in an individual animal or specimen may vary from what is presented in table 1 but should still conform with the information presented in the "Field Criteria for Diagnosis" and "Laboratory Criteria for Diagnosis" sections.

Table 1. Case classification chart for West Nile virus (avian).

[The suspected case classification does not apply to this case definition.]

Individual, place, and time criteria	Field criteria (if available)	Laboratory criteria	Epidemiological linkage criteria	Classification	
Diagnosing West Nile virus (WNV)					
Avian, any place, typically mosquito season	Lethargy, ataxia, blindness, unusual posture, inability to perch or stand, re- cumbency, seizures, and death	Meets laboratory confirmed criteria: Corvids.—Positive PCR, IHC, or virus isolation (confirmed with PCR) with or without gross and histopathology lesions Raptors and other species.—Positive polymerase chain reaction (PCR), immunohistochemistry (IHC), or virus isolation (confirmed with PCR) and gross and/or histopathology lesions Gross examination.—None or may include emaciation, areas of pallor in the heart and pectoral muscles, hepatomegaly and splenomegaly. Absence of other causes of mortality in corvids. Histopathology.—Includes characteristic lesions (mononuclear necrotizing myocarditis or myositis, non-suppurative encephalitis, or meningoencephalitis). May include endophthalmitis, hepatitis, lymphoid depletion of the spleen and the bursa, cryptitis, pancreatitis, or ganglioneuritis. In corvids, no lesions or intestinal necrotizing cryptitis and absence of other causes of mortality. Diagnostic test(s).—Positive virus isolation (confirmed by PCR) or PCR or IHC	Optional: Close geographic or temporal proximity to a con- firmed case, or at a site with a recent history of confirmed WNV	Confirmed	
Avian, any place, typically mosquito season	Lethargy, ataxia, blindness, unusual posture, inability to perch or stand, re- cumbency, seizures, and death	Meets laboratory supportive criteria: Corvids.—Gross and histopathology only. Raptors and other species.—Gross and histopathology only or positive virus isolation (confirmed by PCR) or PCR or IHC only (no gross and histopathology information) Gross examination.—None or may include emaciation, areas of pallor in the heart and pectoral muscles, hepatomegaly, and splenomegaly. Histopathology.—None or may include mononuclear necrotizing myocarditis and myositis, non-suppurative encephalitis or meningoencephalitis, endophthalmitis, hepatitis, lymphoid depletion of the spleen and the bursa, pancreatitis, ganglioneuritis, and intestinal necrotizing cryptitis (in corvids) Diagnostic test(s).—Negative or inconclusive viral culture, PCR or IHC, or testing not done or positive PCR or IHC only (raptors and other species)	Optional: Close geographic or temporal proximity to a con- firmed case, or at a site with a recent history of confirmed WNV	Presumptive	

Case Classification

 Table 1.
 Case classification chart for West Nile virus (avian).—Continued

[The suspected case classification does not apply to this case definition.]

Individual, place, and time criteria	Field criteria (if available)	Laboratory criteria	Epidemiological linkage criteria	Classification	
Detecting West Nile virus (Orthoflavivirus)					
Avian, any place, typically mosquito season	Not applicable	Meets exposed criteria: Gross examination.—None and Histopathology.—None and Diagnostic test(s).—Positive serology	Optional: Close geographic or temporal proximity to a con- firmed case, or at a site with a recent history of confirmed WNV	Exposed	
Avian, any place, typically mosquito season	No evidence of illness suggestive of WNV	Meets present/detected criteria: Gross examination.—None and Histopathology.—None and Diagnostic test(s).—Positive PCR or virus isolation	Optional: Close geographic or temporal proximity to a con- firmed case, or at a site with a recent history of confirmed WNV	Present/detected	

Quality Assurance Review Schedule

The Canadian Wildlife Health Cooperative and the U.S. Geological Survey National Wildlife Health Center staff plan to review this case definition periodically to incorporate new scientific information and test methods as needed.

Planned date for next review.—June 1, 2025 Schedule.— June 2025 and then every 3–5 years—or sooner if science about WNV changes substantially.

Impact

Applying case definitions in diagnostic, surveillance, and research efforts can help standardize data, making it easier to understand and analyze within and between diagnosticians and laboratories. Laboratories are encouraged to store the case classification assigned to each specimen or sample in their data system so that it can be readily and reliably retrievable.

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Glossary

additional diagnostic comments Any additional diagnostic notes pertinent to recording/reporting (for example, requests for strain/serovar/variant reporting, inconclusive/ambiguous results, or "not applicable").

case classification The sum of the factors in the "Case Definition Criteria" sections of the case definition including individual (for example, species, age group), place, time, history, clinical signs, diagnostic observations, and (or) diagnostic test results, associated with a particular disease or pathogen/toxin in an individual animal or specimen. Depending on the confidence in the results, cases of a specific disease will be classified as "confirmed," "presumptive," or "suspected;" and a pathogen or toxin will be classified as "exposed" or "present/detected."

case definition A consistently applied, scientifically based and clearly defined set of field, gross, histopathology, laboratory, or epidemiologic criteria used to classify an individual animal or sample to a specific disease or pathogen/toxin for surveillance or outbreak reporting purposes (based on the combination of the criteria and confidence in the results).

confirmed case The combination of individual (for example, species, age group), place, time, history, clinical signs, and laboratory criteria for diagnosis with the highest level of certainty for accepted diagnostic testing as stated in the case definition. Example: Cardinal with clinical signs, gross and microscopic lesions compatible with salmonellosis, and positive bacterial culture for *Salmonella enterica enterica* in the liver.

diagnostic test(s) Laboratory tests typically used to determine this diagnosis or detect the pathogen/toxin; for example, bacterial culture.

diagnostically compatible An animal that meets the individual (for example, species, age group), place, time, field, and laboratory criteria for a particular disease as stated in the case definition.

disease Any disorder of structure or function that may produce specific clinical signs; disease can be infectious or noninfectious.

disease agent Any pathogen, toxin, or other known cause of disease.

epidemiologically linked A case that has temporal, geographic, or other relevant linkages to one or more confirmed cases as described under "Epidemiologic Linkage Criteria for Diagnosis" in the case definition.

exposed Detection of a toxin in tissues or body fluids at a concentration above acceptable background levels but below the documented lethal threshold level for the species. This may apply to a toxin detected in the absence of documented lethal threshold levels. This category can also include serological evidence of infection in the absence of other information such as organism detection or disease diagnosis.

gross examination Gross necropsy observations in a carcass or sample that are diagnostically compatible with disease.

histopathology General microscopic observations in a carcass or sample that are diagnostically compatible with disease.

history and clinical signs Field observations and changes to behavior, appearance, or abilities in live animals/populations that are diagnostically compatible with disease. Photograph or video evidence may be used when appropriate.

individual The common age groups, species, or other characteristics that increase disease or pathogen/toxin suspicion.

laboratory confirmed The strongest degree of assurance in identification of a disease agent of interest and evidence of the associated disease based on one or more accepted laboratory methods. A test or combination of methods that has been scientifically accepted as definitive for a particular disease agent and the associated disease. Example: Positive bacterial isolation for salmonella plus compatible gross and histologic lesions for salmonellosis.

laboratory criteria for diagnosis The gross, microscopic, molecular, culture, analytical or other laboratory test criteria used to determine the presence of a specific disease agent and evidence of the disease itself. These are categorized based on the validity and performance of the test(s). Categories are "laboratory confirmed," "laboratory supportive," "exposed," and "present/ detected." Where possible, references for the current accepted science for a given disease and pathogen are provided in the case definition. For some select new or emerging diseases the laboratory criteria may be based on the collective expertise of pathologists at the U.S. Geological Survey National Wildlife Health Center and the Canadian Wildlife Disease Cooperative or other institutions.

laboratory supportive Laboratory results that are less than definitive for a specific disease agent and the associated disease. A test or combination or methods whose results support the diagnosis or a particular disease but are not considered definitive; for example, a screening test. Test result interpretation may be based on the tissue tested (for example, culture of amphibian skin surface versus internal tissue) or postmortem condition of the sample. Example: Gross and histologic lesions compatible with salmonellosis (without laboratory testing).

notifiable/reportable disease A disease or pathogen that by law must be disclosed to State, Provincial, and (or) Federal agricultural or public health authorities.

other (field criteria) Additional pertinent comments about presentation (for example, potential for carrier status).

place Locations and other geographic features that increase disease or pathogen/toxin suspicion.

present/detected Laboratory detection of a potentially pathogenic agent in the absence of findings diagnostically compatible with the associated disease. Often used when tracking a known or suspected asymptomatic carrier state (for example, Salmonella or duck virus enteritis) or when documenting detection of an agent that is of increased diagnostic or epidemiologic interest, even in the absence of evidence of illness (for example, new or emerging disease or syndrome).

presumptive case The combination of individual (for example, species, age group), place, time, history, clinical signs and laboratory criteria for diagnosis that has a moderate degree of certainty as stated in the case definition. This uncertainty may be due to the test performed, postmortem decomposition of the carcass affecting observation or interpretation of gross and or histopathologic lesions, inadequate sample for testing due to scavenging or carcass size, inconclusive test results, or lack of a definitive diagnostic test. Enough information is available to conclude the disease is most likely present but not enough information available to conclude the disease is definitively present. Example: Raccoon with compatible histologic lesions for parvovirus without additional laboratory test results.

scope Indicates what species, when and (or) where this protocol applies; for example, specifics regarding the disease agent, animal class, sex, age group, location, season, antemortem or postmortem sample collection, environmental samples, and so on.

suspected case This is primarily based on a combination of individual, place, time, minimal or nonspecific field and laboratory information and a geographic and temporal (epidemiologic) connection to a confirmed case. There is not enough information available to meet the threshold in the case definition for a confirmed or presumptive case, but the diagnosis can reasonably be inferred by the close association with confirmed cases of a particular disease in other animals collected from the same general location and time. Example: A specimen with a geographic or temporal link to a confirmed case of a disease that is not tested but was examined and may have nonspecific gross or histopathologic findings that are compatible with that disease.

time The season(s), months, or other temporal factors that increase disease or pathogen/toxin suspicion.

wildlife Free ranging vertebrate species (mammals, birds, reptiles, amphibians, and fish).

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