Avian Botulism Case Definition for Wildlife

Chapter 1 of
Section E, Toxin
Book 19, Wildlife Disease Case Definitions

Techniques and Methods 19–E1

U.S. Department of the Interior
U.S. Geological Survey

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Conversion Factor

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:
$$°F = (1.8 \times °C) + 32.$$

Abbreviation

PCR   polymerase chain reaction

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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 etiologic agents.

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.—Avian botulism

Pathogen/toxin etiologic agent(s).—*Clostridium botulinum* (van Ermengem, 1896; Bergey and others, 1923) toxin types C or E

Scope of the Case Definition

This case definition applies to all 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.—Likely all avian species, with the exception of vultures, are susceptible to intoxication; notably, increased index of suspicion exists in waterfowl, shorebirds, and gulls for type C, and fish-eating birds such as loons and gulls for type E botulism (Rocke and Bollinger, 2007).

Place.—No restrictions but increased index of suspicion exists in water with eutrophication, decaying vegetation, or decomposing carcasses (Rocke and Friend, 1999; Rocke and Bollinger, 2007).

Time.—No restrictions, but increased index of suspicion exists during summer and early fall. *C. botulinum* grows best under anaerobic conditions at 9 degrees Celsius (type E) and 40 degrees Celsius (type C) (Rocke and Friend, 1999; Rocke and Bollinger, 2007).

Field Criteria for Diagnosis

History and clinical signs.—Ingestion of *C. botulinum* toxin results in an intoxication of variable severity. Diagnostically compatible illness may present with neurological impairments such as the inability to fly, prolapsed third eyelid, and symmetric paralysis in legs and neck that can rapidly progress to death in severe cases. Healthy, sick, and dead birds are commonly observed in the same area. The presence of carcasses can promote the likelihood of botulism spreading through the carcass-maggot cycle for type C (Rocke and Friend, 1999; Rocke and Bollinger, 2007).

Other.—Not applicable.

[1]U.S. Geological Survey.

[2]Canadian Wildlife Health Cooperative.

Laboratory Criteria for Diagnosis

Gross examination.—Diagnostically compatible findings may include good body condition and lack of specific lesions, although body condition can be variable. Signs of drowning or aspiration pneumonia may be evident. Maggots may be found in the gastrointestinal tract for type C (Rocke and Friend, 1999; Rocke and Bollinger, 2007).

Histopathology.—No required lesions.

Diagnostic test(s).—Mouse neutralization test (Lindström and Korkeala, 2006), or polymerase chain reaction (PCR) (Le Maréchal and others, 2017).

Laboratory Criteria Categorization

Laboratory confirmed.—A diagnostically compatible case with a positive mouse neutralization test result of *C. botulinum* toxin in serum from heart blood collected at necropsy in birds that are freshly dead or were moribund at the time of collection (Lindström and Korkeala, 2006).

Laboratory supportive.—A diagnostically compatible case without a positive mouse neutralization test result; or a diagnostically compatible case with a positive PCR result for *C. botulinum* toxin genes (Rocke and Friend, 1999; Rocke and Bollinger, 2007).

Exposed.—Not applicable.

Present/detected.—Not applicable.

Supplemental Diagnostic Information

Additional diagnostic comments.—Toxin type should be recorded in etiology. Birds that were euthanized early in the disease process are less desirable for testing and could lead to equivocal results. Birds that were euthanized with barbiturates are not desirable for testing (traces of the barbiturate remaining in the serum may affect the results of the mouse neutralization test by killing the mice). A positive botulism test from a decomposed carcass (because botulism toxin can be produced after death) or instances in which the protected and unprotected mice both become sick or die are inconclusive findings that do not meet the case classification thresholds for this disease/pathogen (Rocke and Bollinger, 2007).

Notifiable/reportable disease.—Not applicable.

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 avian botulism or at a site with a recent history of confirmed avian botulism with similar presentation as described in the "Case Definition Criteria" sections.

Case Classification

The sum of the criteria listed in the "Case Definition Criteria" sections (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 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. Although an epidemiological linkage may be present in all classifications, the "suspected" case classification requires compatible epidemiological linkage criteria are met. Individual disease and pathogen specific details are presented in table 1.

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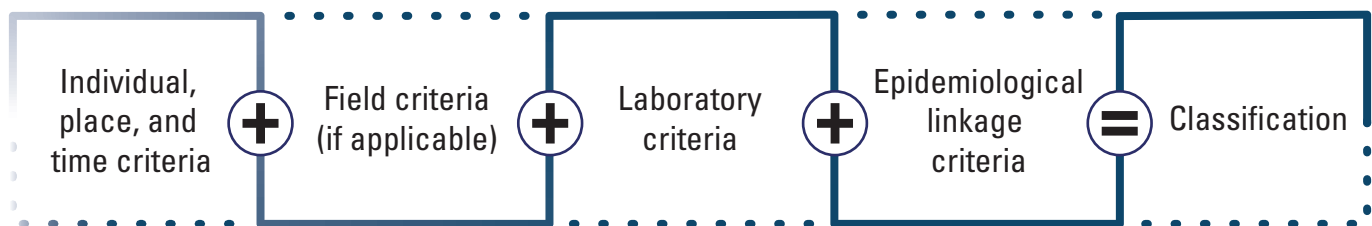

Figure 1.   Case definition criteria add up to the case classifications. From Miller and others (2024).

Table 1. Case classification chart for avian botulism and *Clostridium botulinum* toxin types C and E.

[The detecting toxin/pathogen case classifications (exposed and present/detected) are not applicable to this case definition]

Individual, place, and time criteria	Field criteria (if available)	Laboratory criteria	Epidemiological linkage criteria	Classification
		Diagnosing Avian botulism		
Avian, any place, anytime	Paralysis and good body condition (If bird is found dead then may not have any field criteria information.)	Meets laboratory confirmed criteria: *Gross examination.*—No lesions **and** *Histopathology.*—None **and** *Diagnostic test(s).*—Positive mouse neutralization from moribund or freshly dead bird	Optional: Close geographic or temporal proximity to a confirmed case, or at a site with a recent history of confirmed avian botulism	Confirmed
Avian, any place, anytime	Paralysis and good body condition (If bird is found dead then may not have any field criteria information.)	Meets laboratory supportive criteria: *Gross examination.*—No lesions **and** *Histopathology.*—None **and** *Diagnostic test(s).*— Equivocal mouse neutralization test result and (or) positive PCR results for *C. botulinum* toxin genes	Optional: Close geographic or temporal proximity to a confirmed case, or at a site with a recent history of confirmed avian botulism	Presumptive
Avian, any place, anytime	Paralysis and good body condition (If bird is found dead then may not have any field criteria information.)	Some information but not enough to meet laboratory supportive criteria: *Gross examination.*—No lesions **and** *Histopathology.*—None **and** *Diagnostic test(s).*— No test results (test not performed) or test is negative	Required: Close geographic or temporal proximity to a confirmed case, or at a site with a recent history of confirmed avian botulism	Suspected

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 avian botulism 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. Not enough information is 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).