Mortality Investigation

Chapter 3 of
Section B, Concepts in Wildlife Disease Surveillance, Investigation, and Management
Book 15, Field Manual of Wildlife Diseases

Techniques and Methods 15–B3

U.S. Department of the Interior
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By Thierry M. Work

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Edited by J. Christian Franson, Milton Friend, Samantha E.J. Gibbs, and Margaret A. Wild

U.S. Geological Survey, U.S. Fish and Wildlife Service, and National Park Service

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Introduction

Wildlife mortality events usually occur unannounced and may find management agencies unaware. These events can become highly visible and politically charged affairs, depending upon the scale or species involved. The public, media, and (or) politicians may pressure managers, field investigators, and diagnosticians to quickly identify the cause or to comment on potential causes, the significance of the event, what is being done about it, and a resolution. It may be common during such events for speculation to rage, and for conflicting theories to be advanced to explain either the environmental conditions that led to the mortality or the actual cause of death.

The overarching goal of a wildlife mortality investigation is to determine what happened and to identify causative factors that may have led to the event. Steps include obtaining the relevant history (defined later), conducting on-site field evaluations when necessary, collecting suitable samples, and completing appropriate diagnostic laboratory analyses. These procedures allow diagnosticians and biologists to determine the potential cause of mortality as swiftly as possible, or at the very least, to systematically eliminate potential causes of mortality so as to allow managers to focus on the most probable cause.

Investigating mortalities in free-ranging wildlife poses a series of challenges requiring careful preparation, clear evaluation of objectives, and flexible implementation. The following sections outline these areas in greater detail.

Preparation

Resources

Successful wildlife mortality investigations require access to antemortem samples from sick or dying animals and/or tissue samples from dead specimens, the carcasses of which are still relatively fresh. The more decomposed a specimen, the less value it has to the laboratory diagnostician in terms of informing what could have killed the animal (fig. 1). Obtaining specimens within 24 hours of death is ideal, but in general, the quicker the better for specimens to be collected and delivered to a diagnostic laboratory. Mammals and birds decompose slower than reptiles and amphibians; **invertebrates** and

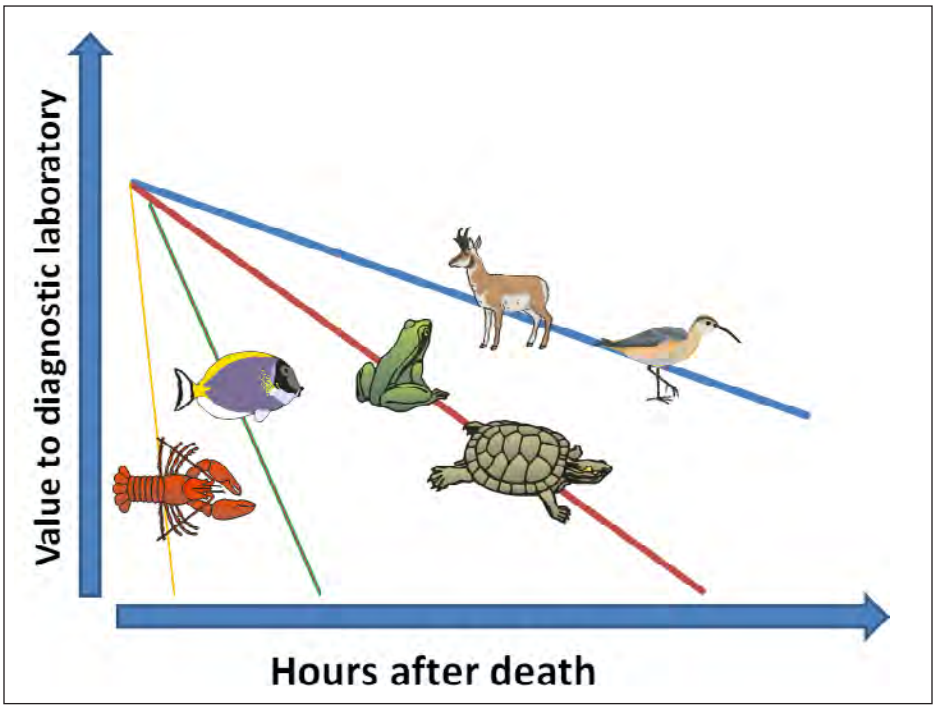

Figure 1. The value of a specimen to a diagnostic laboratory degrades the longer the carcass sits out in the field. This is particularly so in cases where climate is hot and humid. Invertebrates degrade much quicker than mammals and birds. Thus, timely recovery and preservation of specimens is key to aiding the laboratory diagnostician.

fish decompose the quickest and are best collected while still alive. Thus, the key is timely reporting of wildlife mortality events and rapid field response to procure specimens. If animals are euthanized, personnel should follow the current version of the American Veterinary Medical Association (AVMA) Euthanasia guidelines (American Veterinary Medical Association, 2013), and describe the specific method used in the history submitted with the animals for necropsy.

Having wildlife mortality reporting networks in place can make a substantial difference, so educating the appropriate audiences to the importance of observing, reporting, documenting, and responding to wildlife mortality events is critical. Biologists from Federal and State wildlife or conservation agencies, wildlife rehabilitators, and even members of the public can play a constructive role. Prepositioning of basic supplies such as shipping containers, ice chests, ice, sample jars, gloves, and other materials is very helpful. Lastly, having access to a network of laboratories with the necessary expertise to handle wildlife specimens is critical.

Authority

It is highly advisable to have the appropriate regulatory and legal groundwork in place prior to actually responding to wildlife mortality events. Many species of wildlife are protected under Federal, State, or territorial laws (e.g. endangered species), and legal authorization (typically a permit) is required to carry out any activity with those animals. Ideally, the quickest way to accomplish this is to liaise with regulatory authorities who have appropriate permits, and ensure that their staff either directly handle or assist with the handling of wildlife. It also is advisable that all institutions participating in the mortality investigation (e.g. diagnostic laboratories, collaborating academic or non-profit institutions) have the proper permits allowing them to receive and hold specimens from regulated wildlife. For example, if samples are to be shipped internationally (in cases where only specialized laboratories can handle particular tests), then it may also be necessary to secure U.S. Department of Agriculture (USDA) or Convention on International Trade in Endangered Species (CITES) import or export permits. Refer to your agency's/refuge's contingency plan (if one is available) for additional guidance on wildlife disease response.

Expertise

Wildlife mortality investigations are most successful when they are done as partnerships among animal disease diagnosticians and field biologists. Most animal disease diagnosticians have in-depth knowledge about aspects of animal physiology, **epidemiology**, anatomy, **pathogens**, and disease **pathogenesis**. However, understanding causes of wildlife mortality also requires in-depth knowledge about factors such as animal **ecology**, behavior, and habitat use. Diagnosticians generally are not knowledgeable about all the different wildlife species and their habitats, so partnering with biologists who have this knowledge is critical. The diagnostician learns from the biologist key points about life history of the animal that may inform laboratory tests whilst the biologist learns from the diagnostician proper sampling of tissues and general approaches to investigating why an animal dies. Continuous communications and establishing a high level of trust between the two parties is essential because the disciplines often employ different approaches and languages.

Training

Wildlife mortality investigations can require personnel to work in arduous or hazardous field conditions; personnel may be exposed to infectious or hazardous **agents**. Thus, it is imperative that personnel involved in investigations follow appropriate safety protocols and obtain proper training to operate safely in the field and with potentially biohazardous materials. Depending upon the situation, additional specialized training may be required in addition to standard biosafety training. For example, investigations of diseases in bats may require specialized training in caving whereas investigations of mortalities in marine animals may require small boat training or even self contained underwater breathing apparatus (SCUBA). For certain activities (e.g. boating, SCUBA), it may be necessary to have letters of reciprocity in place so that personnel from one organization can operate using another organization's equipment. If samples are to be shipped, the receiving laboratory should be contacted, because appropriate training on packaging and shipping of specimens may be needed.

Coordination

Depending upon the scale of the mortality event, the need may arise to coordinate various groups and agencies over large geographic areas. In such cases, it is wise to have in place incident-command systems with clear understanding of lines of authority and responsibility of involved parties *before* the event. The coordinator of large scale wildlife mortality event investigations should have well-tuned biopolitical antennas, be well versed in communicating effectively with a wide variety of stakeholders, and maintain focus on the major objectives of the investigations. Consideration should be given to designating a central point of contact to handle media inquiries and to provide regular press briefings, if warranted.

Is This a Legal Case?

In some instances, wildlife mortalities are part of criminal investigations, and these are termed legal cases. Determining *a-priori* whether the mortality is part of a legal case will determine the degree of documentation necessary to complete the investigation. Covering all aspects of legal case investigations is beyond the scope of this chapter. Determination

of legal status and how to proceed should be done in close coordination with the relevant Federal, State, or territorial law enforcement agencies to ensure that personnel adhere to proper documentation of actions, specimen collections, testing, reporting, and other activities. For example, certain law enforcement agencies may require detailed chain of custody forms, have particular requirements for sample curation or disposal, or identify particular laboratories to which samples should be sent. Depending upon the situation, it may be necessary to balance information provided to the media with the need to maintain the integrity of the legal investigation.

Steps of a Wildlife Mortality Investigation

Wildlife mortality investigations generally have four steps: (1) obtaining a field history; (2) collection of tissues from sick or dead animals (necropsy); (3) laboratory analyses; and (4) communication of results to stakeholders and (5) implementation of appropriate management actions. The ideal outcome of such an investigation is to arrive at the cause of the mortality event. However, for many wildlife species, the field or laboratory tools to do this rapidly may not exist. Thus, it is highly likely in some cases that the cause of death may not be determined. However, if proper diagnostic approaches are followed, what did not cause the mortality event can be determined with reasonable certainty. This then provides a-priori knowledge that allows investigators and managers to focus future efforts on causes to be ruled out and, eventually a cause of death is determined (fig. 2).

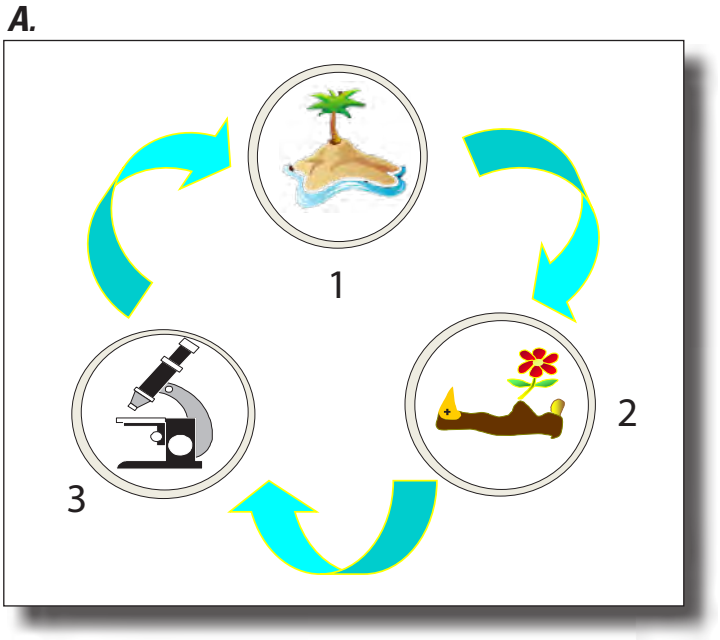

Figure 2. *A*, Investigation of wildlife mortality events begin with (1) field observations, which provide initial clues. These are followed by (2) necropsies and tissue procurement with microscopic examination of tissues that then (3) guide laboratory and biodetection efforts. *B*, In cases where cause of death is not initially determined, this process can eliminate potential causes, and when applied over time, can usually help determine cause of death.

Field History

The purpose of the field history is to collect information about the event that could help the diagnostician rule in or out possible causes of death. Proper documentation of field histories also can facilitate larger epidemiologic investigations, because data that are rigorously collected, stored, and analyzed gain value in the longer term. Collaborating with biologists in this phase is absolutely critical as they have the most intimate knowledge of the animals and habitat and are best equipped to alert the diagnostician to unusual patterns in habitat or animal behavior that could provide important clues. For example, a mortality event involving multiple species might guide the diagnostician toward a non-selective cause of death, such as poisoning, whereas an event involving a single species might indicate a more selective cause of death such as an infectious agent. Factors to consider when obtaining a field history include details about the extent, onset, and duration of the outbreak; species, sex, and age classes of animals affected; clinical signs manifested by sick animals; and species not affected by the event (Friend and Franson, 1999, p. 3–6; SCWDS, 2006). Environmental factors should be recorded such as unusual weather, changes in land use by animals, changes in habitat management, or distribution of affected animals on the landscape. Photographs of the area and close up photos or videos of sick or dead animals can be very informative and provide important clues as to cause of death.

Sample Collection

Many things can kill wildlife including both infectious and non-infectious causes. Hopefully, the field observations provide valuable insights, but diagnosticians still must be able to rapidly and systematically sift through a myriad of possibilities to arrive at the cause of death using a systematic approach. Typically, diagnosticians will begin with a necropsy and describe changes in tissues at the gross and cellular level (histopathology), because this helps guide laboratory investigations. For example, if the diagnostician sees spots on the liver and microscopic evidence of cellular damage associated with bacteria, then the subsequent course of action is to try to identify the bacterium using appropriate laboratory tests. Collecting fresh tissues suitable for laboratory assays is challenging (see "Resources" above); coordinating with field personnel to notice, report, and collect fresh carcasses is really important. If collecting samples from live animals (e.g. blood, swabs, biopsies), then coordinating with personnel who have familiarity with capture and handling of the wildlife species in question is essential to ensure the safety of personnel and animals. Certain situations, such as sampling animals underwater (e.g. marine invertebrates), can pose unique hazards and should be performed only with proper training and certifications. For more details on examples of specimen collections, see Friend and Franson (1999, p. 7–12), SCWDS (2006), and the following links.

Coral

http://www.nwhc.usgs.gov/hfs/Globals/Products/Collecting%20corals%20for%20histopathology.pdf.

Fish

http://www.fws.gov/policy/aquatichandbook/volume_2/chapter4necropsy.pdf.

Turtles

http://www.nwhc.usgs.gov/hfs/Globals/Products/Turtle%20manual%20english.pdf.

Birds

http://www.nwhc.usgs.gov/hfs/Globals/Products/Sea%20bird%20manual%20english.pdf.

Marine Mammal

http://cmsdata.iucn.org/downloads/marine_mammal_necropsy.pdf.

Terrestrial Mammal

http://vet.uga.edu/oldvpp/programs/afvet/attachments/how_to_necropsy_a_mammal.pdf

Laboratory Analyses

A detailed discussion of laboratory tests available to diagnose wildlife diseases is beyond the purview of this chapter. However, field personnel should be aware that coordinating with participating laboratories is absolutely essential to ensure that specimens are properly collected and shipped. Some laboratory tests require particular handling and packaging of samples. Under no circumstances should samples be sent to a diagnostic laboratory without contacting them first. Importantly, not all diagnostic laboratories are the same. Some laboratories only focus on domestic animals or specific tests, while others have a broader scope and will try to determine cause of death. Some laboratories are fee-for-service, and others will do limited work gratis. Some laboratories have rapid turnaround times whereas others may take months to provide results. Field personnel should consult with appropriate agency wildlife disease specialists and maintain a list of laboratories available for and capable of assisting with wildlife mortality investigations.

Communications

Wildlife mortality investigations have a tendency to generate large amounts of field and laboratory data. Often, laboratory results are reported in language and jargon that means little to wildlife managers or decision makers. Thus, it is imperative that those coordinating a wildlife mortality response be able to interpret and communicate laboratory findings to stakeholders in real time in a way that is understandable. This process helps build trust between laboratory and field personnel and ensures a positive feedback loop for good communications and collaborations.

In summary, wildlife mortality investigations pose a series of technical and communication challenges, but if these are overcome, the findings from such investigations can assist management actions, lay the groundwork for more detailed epidemiologic studies, and sometimes lead to profound new discoveries and insights that can aid wildlife management and conservation.

Acknowledgments

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Friend, M., and Franson, J.C., eds., 1999, Field manual of wildlife diseases—General field procedures and diseases of birds: U.S. Geological Survey, Biological Resources Division, Information and Technology Report 1999–001, 425 p.

Davidson, W.R., ed., 2006, Field manual of wildlife diseases in the Southeastern United States: Athens, Southeastern Cooperative Wildlife Disease Study, University of Georgia, 448 p.

Glossary

A

Agent (infectious) A living organism capable of invading another; for example, a bacterium or virus.

Antemortem Before death.

B

Biopsy A test involving the removal of cells or tissues from a living subject for examination, usually by microscope, to determine the presence or extent of a disease.

E

Ecology The study of the interrelationships between living organisms and their environment.

Epidemiology The study of the causes, occurrence, and control of disease in populations.

I

Invertebrates Animals lacking a spinal column; for example, insects, crustaceans.

N

Necropsy The methodical examination of the internal organs and tissues of an animal after death to determine the cause of death or to observe and record pathological changes.

P

Pathogen Typically, a microorganism capable of inducing disease, but broadly including all disease-inducing agents. (*See* also agent.)

Pathogenesis The progression of agent-caused events within the body following exposure to a pathogen (disease agent) that results in disease, morbidity, and (or) death. (*See* also agent, pathogen.)

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