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Recording and Submitting Specimen History Data

Chapter 3 of
Section C, Techniques in Disease Surveillance and Investigation
Book 15, Field Manual of Wildlife Diseases

Techniques and Methods 15–C3

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

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By Barbara L. Bodenstein

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

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Conversion Factors

Inch/Pound to International System of Units

Multiply	By	To obtain
	Length	
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as

$$°C = (°F - 32) / 1.8.$$

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Introduction

With an increased focus on wildlife disease issues in recent years, wildlife disease specialists and wildlife diagnostic laboratories have become more common: however, the role of field biologists in the diagnostic process is also of equal importance. Field professionals are the eyes and ears of the wildlife disease world because of their proximity to wildlife mortality events and their intimate local knowledge of an area. Wildlife mortality events need to be discovered before they can be investigated; therefore, the entire investigatory process begins with the astute field biologist. The local biologist, disease expert, and laboratory diagnostician constitute a team that must work in concert to successfully carry out a wildlife disease investigation. During a disease outbreak, recording a thorough history combined with astute field observations are critical for a complete understanding of the natural history or epidemiology of a disease. By collecting as much information as possible about the species and environment, including detailed descriptions of sick and dead animals, relative locations, and total numbers (solid estimates in the case of massive events) can aid wildlife professionals in determining the most likely responsible agent(s). A checklist of field data collected during a mortality investigation (fig. 1) is provided as an example. Obtaining a sound history of previous disease events in an area also may be valuable. Detailed follow-up field observations during the course of a die-off are also very important to determine the time line of an event. All these data collected in the field can be just as important as laboratory test results in ultimately determining the cause of an event. The combination of field and laboratory information helps managers and disease experts formulate disease response and management plans. The information obtained during a wildlife disease event also becomes a historical record that can be referenced during future events. It is, therefore, crucial to maintain this information in a retrievable format in a local database as well as in regional or national databases (https://www.nwhc.usgs.gov/whispers/).

Before responding to a mortality event, wildlife professionals should first consult with their respective State and Federal wildlife disease experts to discuss response options. These wildlife disease experts are able to provide guidance on health risks, authorities needed (such as permits), specimens to collect, how to collect and best preserve specimens to maximize their diagnostic value. Wildlife disease experts can help formulate a coordinated response and, depending on the size and severity of the event, may provide additional field resources. Personal safety is a primary consideration when responding to a morbidity or mortality event. Field biologists should consider that there may be a potential risk to personnel of exposure to zoonotic agents and hazardous non-infectious agents; therefore; response personnel should have appropriate training, authorization and proper personal protective equipment (PPE) available. Biologists should refer to the health and safety guidelines for PPE for their agency.

What Information Should Be Collected

What may seem insignificant in the field during an event may potentially be the key to a diagnosis; therefore, when collecting field data is important to be as complete and thorough as possible. After discovery of a wildlife mortality event, take some time to think through a response and make an action plan. A simple outline is helpful. Avoid any preconceptions that may limit the information collected and that may bias the investigation. Avoid the “oh, I’ve seen this before and so I already know what this is” way of thinking. One of the most important reasons to investigate wildlife disease events is to understand the disease trends and potential impacts at the population level. To do this, wildlife disease experts need to know the frequency of similar events involving the same species or different species in the same area over time. The frequency of wildlife disease events is not accurate if events are not reported because the local biologist makes a field assumption rather than a diagnostic confirmation. At the very least, make sure to document the event and save the information in a local database.

The “history” is the local current observational information about an event combined with whatever historical information there is about past events. Who, what, where, and when are basic questions that should be pursued and provided in the history. All of the pieces of information outlined here are used along with necropsy findings and diagnostic test results to determine the cause of the outbreak and considerations necessary to make informed management decisions in response to the situation.

Mortality Investigation Data Checklist

COLLECTION INFORMATION

- ☐ Name and contact information of individuals involved in collecting information
- ☐ Estimation of morbidity/mortality onset date
- ☐ Species involved
- ☐ Estimated numbers dead by species
- ☐ Numbers known dead by species
- ☐ Estimated numbers sick by species
- ☐ Numbers known sick by species
- ☐ Clinical signs (for example, unusual behavior or physical appearance)
- ☐ Ages (for example, juvenile/adult)
- ☐ Sexes
- ☐ Dates of detection, carcass pick up(s) and sampling date(s), location/s
- ☐ GPS Coordinates (if available)
- ☐ County of collection
- ☐ State of Collection
- ☐ Land use and human activities
- ☐ Specific features of habitat/area
- ☐ Environmental factors (for example, weather conditions surrounding outbreak)
- ☐ Population(s) at risk (that is, contextual information about species present at the site)

Additional field observation information ______________________________

LABORATORY INFORMATION

- ☐ Number of carcasses by species collected and submitted
- ☐ Preservation methods
- ☐ Laboratory findings
- ☐ Laboratory results and diagnosis
- ☐ Laboratory personnel or diagnosticians conducting the work
- ☐ All other relevant case related notes and comments

Additional observations (for example, whether animals seen prior to outbreak appear undernourished or healthy) ______________________________

Figure 1. Information checklist commonly collected during a mortality investigation.

Complete history and coordination of appropriate specimen shipments should be discussed with the designated point of contact at the diagnostic laboratory prior to shipping, as there is variability in how samples and information are collected, packaged, and what specific information is required on laboratory submission forms. These standards often differ by laboratory, so having this information on hand before an event can save time and prevent misunderstandings, as well as lost specimens. A paper copy of the history should be sent along with specimens to a diagnostic laboratory. This recorded history report requirement has become increasingly necessary as a result of enhanced laboratory security requirements. History reports specific to wildlife mortality events and standard shipping instructions for USGS National Wildlife Health Laboratory are provided as examples at the following links: http://www.nwhc.usgs.gov/services/Wildlife_Mortality_Reporting_and_Diagnostic_Services_Request_Form_120415_saveable.pdf and http://www.nwhc.usgs.gov/mortality_events/shipping_instructions.pdf.

Mortality Event Location Information

Describe the location of a die-off event so that a relatively specific area can be identified on a map. Keep in mind that the event could span a large geographic area and additional reconnaissance may be necessary. At a minimum, the field observer should record the nearest town or cross road, county, and state in the history. As part of the specific area description, include any available precise location data such as global positioning system (GPS) locations. Sometimes there will be concerns about proprietary location information or private land access issues that may complicate providing specific location information. It is important to recognize these issues and provide location information based on sensitivity of data. Sensitive data may have different levels of restrictions based on the person/group receiving the data and therefore field biologists may need to refer to their agency's policies.

Environmental Factors

Determine if the start of mortality coincided with any unusual event. When investigating a mortality event, consider both biotic and abiotic features that could have influenced a disease. Environmental changes such as rapid or drastic weather changes, storms, precipitation, droughts, or changes in habitat quality or management are potential sources of stress for wildlife that can trigger mortality events. Record any recent weather changes in temperature or precipitation or if the preceding season was unusually dry or wet, followed by the opposite. A food shortage may degrade the body condition of wildlife and increase their susceptibility to disease or force animals to forage on atypical food sources (Wobeser, 2006). Water-level changes may concentrate or disperse birds, for example, alter the accessibility of toxins in food or water, or cause invertebrate die-offs thereby affecting food availability (Wobeser, 2007). Information on sucking and biting insect population numbers and presence may indicate possibility of blood-borne infections in many species (Wobeser, 2006).

The quality of the water used as a source for an impoundment may contribute to disease or mortality (Wobeser, 2007). Changes in water levels such as severe flooding, drawdowns, or drought, can modify habitat availability, quality, and use (Wobeser, 2007). For example, poor water quality may provide anaerobic conditions that contribute to avian botulism or may be a primary cause of mortality if water is contaminated by toxic substances (Friend and Franson, 1999). Note any unusual water odor, colors, or presence of algal growth. Some algae produce toxins that can kill quickly, whereas others take more time. Petroleum oil products can affect the integrity of feathers and may be toxic if ingested. Record recent pesticide applications, other habitat or crop management practices and any previous disease problems in the area. Include in the history any known recent catastrophic events, such as, but not limited to chemical spills, volcano eruptions, severe storms, microbursts, temperature inversions, wildfires, drought, blizzards, and flooding.

Date Specimens Collected

If there are multiple collection dates then indicate respective dates on each specimen (body tag), corresponding laboratory data sheet, and field tracking log. Repetitive site visits and carcass collections are important because the quality of specimens change over time during a disease outbreak. Specimens collected and frozen over time commonly have less diagnostic value than recently collected freshly dead animals; however, this is not absolute. Long-term frozen samples, in some cases, are the only samples available (White and Dusek, 2015).

Submitter

Provide the name and contact information for the primary point of contact for the specimens being submitted. Ideally, the submitter should be affiliated with the responding wildlife or land management agency, such as a State, Federal or Tribal wildlife agency staff or their designee, as these agencies have management authority for the species involved or the lands the event is occurring on.

Collector

Provide the name and contact information of the field collector if different than the submitter. Examples may include, but are not limited to, agency staff (with no regulatory authority over species or lands), university researchers, wildlife rehabilitators, beach survey crews, municipality staff or the general public.

Method of Collection

It is important to collect representative specimens found, by species, age and in various postmortem conditions. For example, carcasses of animals that died at the site may have a different cause of disease than an animal that was captured alive at the site and euthanized. The manner in which each specimen was collected should be recorded for each of the individual specimen histories (found dead; found alive and died while being handled; or found alive and euthanized) and should be included on the carcass tag. If lethal collection is considered necessary, biologists should refer to the policies, procedures, and permit requirements of their institution/facility and the agency responsible for species management (U.S. Fish and Wildlife Service or State natural resource agency) before lethal collection is used in the field (White and Dusek, 2015). In some instances, an animal that dies during handling may provide more important clues to determine the cause of death than an animal euthanized or found dead. If multiple types of carcasses are available, submit a range of found freshly dead and found sick (and died in hand or euthanized). If euthanasia is required, the precise method used should be clearly stated on the specimen form. For example, an animal that was euthanized by gunshot may be diagnosed with gunshot trauma and no further investigation completed, if the submitter fails to report this important information. Biologists should also be aware that some methods of euthanasia may interfere with diagnostic laboratory evaluations. For example, avoid gunshot to the head if you suspect neurologic problems, as the brain will need to be closely examined during diagnostic evaluation. In addition, some methods, such as chemical euthanasia may require special training and a veterinary license for possession of required chemicals/drugs, may pose human health and safety risks, and may require special carcass disposal procedures. Safe handling protocols to prevent injury or disease transmission to handlers should be available.

Specimen Preservation

It is important for the receiving entity to know how individual specimens were collected and what preservation method is used for each specimen before shipping. For example, note the post mortem condition of animal carcasses collected in the field, and the length of time chilled or frozen in preparation for shipment. As a general rule, if a mammal, bird, or reptile carcass cannot be shipped in a chilled condition and arrive at the designated laboratory within 48–72 hours post collection, freezing is generally recommended until samples are shipped to the diagnostic laboratory. Consult with appropriate wildlife disease specialists or diagnostic laboratory before preservation as needed.

In some instances, moribund amphibians should be considered to obtain meaningful bacterial cultures and most types of fungal cultures. Consultation with the submitting agency's institutional animal care and use committee before collection may be warranted. If live animals are not available or are not authorized, chilled or frozen specimens would be the best alternative. For more specific details on carcasses or specific specimens, see Chapter C4 Wildlife Specimen Collection, Preservation and Shipment (White and Dusek, 2015). Also refer to Chapter B3 Mortality Investigation, figure 1 for more details on carcass degradation over time (Work, 2015).

Estimating Disease Onset

When estimating the onset of disease, several factors should be considered: (1) the earliest date when potential causal factors such as storm events, tidal movements, human activity/disturbances could have contributed to or resulted in sick or dead animals; (2) the actual date when sick or dead animals were first detected and the date someone last visited the site. For example, is the area surveyed once a week, once during breeding season, or are there remote cameras on site that capture daily activity; (3) the proportion of freshly dead carcasses compared with the number of scavenged and decomposed carcasses. The abundance and types of scavengers and predators can predict how long carcasses remain in the area. Low levels of predation during a mortality event may prevent detection until carcass availability exceeds predator consumption rate (Wobeser, 2006). For example, very few sea otter or sea bird beach cast carcasses may be noticed in a morbidity/mortality event because of high foraging activity by predators and scavengers. Tidal movements may also prevent notice of a morbidity/mortality event. In events with decomposed carcasses, other useful information about the onset of the mortality event can be gained from recording any differences in plumage, including stage of molt, if present, between live and dead birds. Size differences between live and dead nestlings/fledglings in birds, siblings/litter mates in mammals, metamorphose stages in amphibians within family and cohorts may also provide useful information for comparison with known growth rates. Consider how air, water, and soil temperatures affect the speed of decomposition. The higher the temperatures; generally, the more accelerated the decomposition of tissues and fly/maggot activity (Wobeser, 2006). Include these observations in the history.

Species Affected

Much can be learned by recording all the species present and whether they appear healthy, sick or dying in the immediate vicinity of an event. This includes information about the surrounding area. For example, are there other ponds in the vicinity of an amphibian die-off? Are other nearby ponds affected or not? If this is a large waterfowl die-off on a lake what is happening in the lakes in the surrounding area? In many larger wildlife species, sick animals are harder to observe because they typically hide. The first discovery likely would be dead animals so finding sick animals will take greater effort. Those species present but unaffected can indicate a narrow host range, whereas other diseases such as avian cholera, biotoxins or toxicants affect a wider host range. Mortality in avian or mammalian scavengers in addition to the affected species suggests the agent may be spread through ingestion of affected prey or carcasses. In aquatic environments, dead fish, amphibians, reptiles, fish-eating birds, mammals, and invertebrates are important to note, as they are generally associated with a biotoxic event such as botulism, harmful algal blooms, and other toxins (Wobeser, 2007).

Age

It is important to determine the ages of animals involved in a mortality event. This is especially true if the disease causing agent is not previously known and is newly discovered in an event. Some disease agents may kill young animals but leave adults unaffected because of age-related disease resistance; other diseases kill animals of all ages, although young or old animals may be more susceptible because of additional stress placed on these age groups (Wobeser, 2006).

Sex

Differences in mortality rates by gender may be apparent in colonial nesting birds when females are incubating eggs and therefore in close proximity or in other situations where the sexes are segregated. Gender differences in foraging behavior of manatees resulted in biasing male mortality rates incorrectly suggesting that males were more susceptible to brevetoxin than females. In fact, males were traveling greater distances away from warm water refugia into brevetoxin rich areas to feed. Females were not traveling to the same areas (Scott D. Wright, written commun., 2014). Understanding the biology of the species involved in an event is crucial to understanding the event.

Number Sick/Number Dead

The proportion of sick versus dead animals can help determine the disease cycle. Many sick animals with a few dead may potentially indicate the event is discovered early in the disease cycle or the disease is less virulent. The overall proportion of dead/sick animals will increase over time, but in some diseases a high number of sick animals may remain for some time. As the outbreak nears the end, the number of sick and freshly dead animals will usually decline. Determining and tracking the numbers of animals involved over time (disease cycle) underscores the importance of follow-up surveillance and monitoring in the field after the initial discovery of an event. It is also useful to set the appearance of a number of carcasses as background, so that finding more carcasses than the background number is a “trigger event” indicating that something is going on. Establishing a threshold number is especially useful in situations where the animals are closely monitored (endangered species) or when routine surveys (such as beach cast surveys) are completed.

Clinical Signs

Clinical signs for many diseases in live animals will simply be listlessness and being nonresponsive to disturbance. Whereas clinical signs associated with other diseases may be more dramatic such as twisting the head or neck, paralysis of wings or legs, staggered gate and convulsions. Try to be as descriptive as possible about any unusual behaviors, sounds or body changes (clinical signs) you observe. Describe unusual behaviors in a sick animal such as lack of normal response to being approached, tremors, vomiting, or the animal making audible sounds such as coughing and unusual vocalization. Video or photographs can be very helpful, but they should not take the place of a written description of clinical signs. The written description will become part of the historical record of the event, whereas photographs and videos are less likely to be saved.

Population at Risk

The population at risk is the best estimate of the number and species that may be present and vulnerable. Remember that the animals you see sick and dead may not be the only animals at risk. Disease specialists need to have an idea of what and how many animals potentially are being exposed to the disease agent to make appropriate suggestions for disease management. Please be more specific than saying “there are a lot of waterfowl present” or “it is a big herd”. You may have to rely on ground counts or aerial surveys. If good historical records of the species levels and activity patterns are available for the area in question, then the most effective locations and time periods for surveys for sick and dead animals may already be known. If these data are not available from historical records, then the initial disease surveillance effort may involve wide-ranging and intensive surveys of the entire area to obtain the animal counts and activity data needed to narrow down the survey area and develop the most efficient monitoring methods. If surveys are taken regularly, provide a copy of the most recent data, along with what is expected for that time of year. Contact local wildlife managers to determine any threatened or endangered species that may be at risk, as special management for collection and handling permits may need to be implemented.

Population Movement

Record recent change in population, as well as other species present. If species numbers have recently changed, try to determine a movement history of the animal. Knowing what normal movements are during different seasons will help identify when abnormal situations arise. This information may be gathered when population movements are being monitored for census, hunting forecasts, and other purposes. Other natural resources managers or landowners are good primary sources of information to aid in the investigation and response.

Specific Features of Problem Areas

Describe the problem area in detail so someone with no knowledge of the area can visualize major characteristics, such as topography, soil, vegetation, climate, water conditions, and land use. Maps or aerial satellite images can be used to identify locations of groups of dead animals and any differences of habitat where they are found. Animals found in agricultural fields may be dying of pesticide exposure, animals with more chronic toxicoses usually seek dense cover, and animals dying of acute diseases may be found in a variety of situations. Check any relation between specific animal use patterns and the location of affected animals, such as roost sites, loafing areas, and feeding sites.

Example Description of Problem Area

The problem area is a 10-acre freshwater spring fed pond located in Teno County, North Carolina, 1/2 mile east of County KV, 5 miles north of Highway 43. The pond has an average water depth of 6–12 feet and a sandy substrate. Vegetation around the pond border is bull brush and reed canary grass. The surrounding uplands are essentially flat for one-half mile in all directions and lie fallow, covered with grasses and some shrubs. The area affected is along the coast with enough relief to prevent saltwater intrusion into the pond even during major storms. Weather for the past 2 weeks has been pleasant and there has been no precipitation. Daytime temperatures are currently in the mid-80s (°F) and evening temperatures in the 70s. This is an isolated body of fresh water with good clarity, and sustains a few hundred waterfowl, gulls, and small numbers of wading birds and shorebirds, and healthy fish and amphibian populations. Cattle graze the adjacent area. There does not appear to be an overabundance of algae or aquatic vegetation. There are no residential or industrial buildings within 1 mile of the site. Human visitation is frequent for bird watching, fishing, and hiking. Companion animals such as dogs are allowed on the area.

Photographs and Videos

Photographs and video taken over the course of an event can be helpful evidence that captures habitat, environmental conditions, clinical signs and gross lesions, various behaviors of sick animals, or unique body changes associated with a mortality event. Social media can be a useful tool to gather observations from visitors. For example, many researchers now (2016) use remote cameras to monitor sites such as bird staging areas, visitor areas, nests and dens. These remote camera recordings can become invaluable to capture clinical signs in live animals, weather conditions, and other factors leading up to mortality.

Followup to Initial Investigation

In many instances it may be necessary to monitor the mortality site over time to obtain all of the pertinent information. Monitoring should be completed once specimens have been collected and submitted to the appropriate diagnostic laboratory. Summarize the findings and observations of monitoring in a supplemental report that includes dates, species, numbers observed, and an estimated date that the mortality ceases or subsides. Maintain a copy of the new report in station files, and provide a copy to the diagnostic laboratory where the specimens were sent. Reports should contain the dates of investigations, time of day, weather conditions, number of investigators, methods used such as air and ground searches, time spent on the investigation, and results.

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Supplementary Reading

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