TECHNIQUES FOR WILDLIFE INVESTIGATIONS AND MANAGEMENT

Edited by

Clait E. Braun

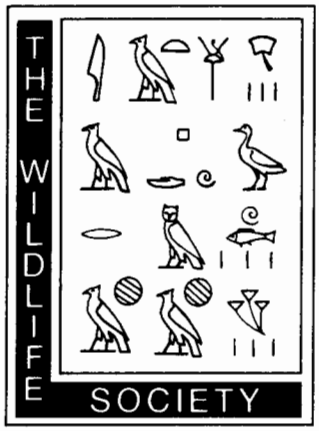
Grouse Inc.

and

School of Natural Resources

University of Arizona

Tucson, Arizona



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WILDLIFE HEALTH AND DISEASE INVESTIGATIONS

Thomas J. Roffe and Thierry M. Work

BACKGROUND

Wildlife diseases are increasingly important in wildlife conservation, particularly when endangered species or human health is involved (Daszak et al. 2000). Of 175 newly identified infectious threats to humans, 132 (75%) are zoonoses (diseases that can be transmitted between humans and animals) (Taylor et al. 2001). Overall, zoonotic pathogens are twice as likely to be associated with emerging human diseases than nonzoonotic agents (Taylor et al. 2001). Recent national concerns over West Nile virus, chronic wasting disease, and tuberculosis and the continuing concern over rabies and Lyme disease have sensitized people to the role wildlife may have in their own well-being. Timely and efficient investigation of wildlife diseases is also important for implementation of effective management for mitigation or prevention of further wildlife losses. However, biologists often ignore wildlife mortality episodes for several reasons. Diseases are often considered part of the "natural" ecology. Many biologists are unfamiliar with exactly how to approach and resolve wildlife mortality issues, or have the perception of "why bother?" because little can be done about wildlife mortality. This is unfortunate, because mortality, like recruitment and immigration/emigration, is 1 of the 3 fundamental demographic factors that affect success or failure of wildlife populations. Gaining a better understanding of this demographic component can help biologists more effectively manage populations, particularly when it becomes apparent that certain management methods enhance wildlife diseases. Furthermore, some wildlife diseases can be managed with the most basic of methods.

Even if the perception is that disease can be important, biologists frequently ask "when is disease important?" and "when is intervention warranted?" The definition of disease is varied and dictionaries provide a broad definition of a "condition that impairs the performance of a vital function." We prefer a definition of disease as "a disturbance that inhibits biological systems returning to homeostasis." Thus, with these broad definitions even something as simple as a broken leg would be classified as disease. A broken leg would be significant to that specific individual, but would it warrant intervention? The answer depends on your perspective. We suggest that several perspectives be considered in making a decision as to whether intervention is warranted. On the ecological scale, disease that causes mortality affecting population characteristics, perhaps even population survival, may present an unambiguous signal for intervention by most biologists and wildlife management agencies. Another perspective, though, might value conservation of "natural systems" exclusive of human intervention. Thus, low-level endemic diseases that do not threaten populations and are not the consequence of human activities might not warrant intervention. However, under this perspective, a disease that does not threaten population survival but is caused by, or exacerbated by, human wildlife management may warrant intervention.

Today wildlife is frequently managed on small, segregated tracts of habitat. Such management may lead to disease exacerbation (e.g., avian cholera or botulism) that may warrant intervention. Other perspectives are equally valid for some people and should be considered in making a decision whether to intervene to control disease. These may include considerations for public relations and public education regarding wildlife ecology, involvement of endangered/threatened species where loss of a single individual may be important, diseases that are economically important to domestic animals, diseases transmissible to

humans, exotic (not native to the area of occurrence) diseases, diseases poorly characterized for their effect on populations, etc.

Decisions on disease intervention are based on a multiplicity of factors involving biology, politics, economics, resources, mission, management philosophy, and other perspectives. The simplistic view of only intervening when a disease is known to have a detrimental effect on populations is in the minority. There would be no need for the multiplicity of wildlife management agencies managing for different values if there was general agreement in the public at large for a single wildlife management perspective. Regardless of perspective, the only way to make informed decisions about intervention is to have a knowledge base of existing diseases, their prevalence and distribution as well as information on their effects on wildlife.

There are many general references available for biologists to learn about the broad array of wildlife diseases. The broadest general references include Wobeser (1994), Fairbrother et al. (1996), and Williams and Barker (2001) on wildlife diseases, noninfectious diseases of wildlife, and infectious diseases of wild mammals, respectively. Other references cover a wide breadth of species in specific geographic areas, such as Davidson and Nettles (1997) (southeast United States), Dieterich (1981) (Alaska), Thorne et al. (1982) (Wyoming), and Fowler (1981) (Pacific Basin). However, the diseases described in these references are applicable to those species across a much wider geographic area. Some references are restricted to certain species groups (Wobeser 1981, Geraci and Lounsbury 1993, Friend and Franson 1999, Noga 1999). Adrian (1992) provided background on forensic techniques and guidelines for evidence handling of law enforcement cases.

Wildlife diseases and mortality are most effectively investigated when there is a close partnership between wildlife disease specialists and biologists. Wildlife disease specialists are scientists trained in biomedical and epidemiological techniques applicable to wildlife populations. During wildlife mortality investigations, the wildlife disease specialist provides the disease expertise, access to specialized laboratories, and ability to interpret diagnostic findings for the biologist. The biologist has expertise on the habitats of the animals, and their ecology and behavior, all of which can provide valuable clues to cause of the mortality.

Biologists are often the first to observe dead and dying wildlife in free-ranging populations and frequently have background information on recent events that may have affected the population in question. This partnership between wildlife disease specialists and biologists is critical to successfully addressing the impacts of disease on wild populations. The interrelationship between field and laboratory findings to eventually arrive at a diagnosis and plan a strategy for management cannot be overemphasized. Each provides pieces of a puzzle; neither provides the complete picture. To be effective, this process requires a rapid dissemination and 2-way exchange of information between biologists and disease specialists. Such communications build confidence, efficiency, and accuracy.

Where can biologists turn for assistance on wildlife mortality events? In the United States and Canada, biologists have access to multiple resources and organizations that can provide technical assistance on wildlife health related issues (Box 1). These organizations provide a good starting point for inquiry. However many other local, state, provincial, and regional sources of technical and diagnostic assistance exist and biologists are encouraged to develop local relationships. Assistance provided can range from phone consultations to on-site assistance by a wildlife disease specialist. However, because many wildlife mortality events occur in remote areas, it is likely the biologist will need to take the initiative and actively participate in the investigation including taking field samples and notes, discussing management options, and collaborating with the disease specialist to evaluate laboratory findings. This process will be more productive if the biologist is conversant on methods used to investigate wildlife diseases and the wildlife disease specialist is conversant on ecology of wildlife resources being affected.

Box 1. Examples of national and regional wildlife health programs in North America.

UNITED STATES

- National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711. Tel: 608-270-2400; Fax: 608-270-2415.
- Southeast Cooperative Wildlife Disease Study, The University of Georgia, Athens, GA 30602-7393. Tel: 706-542-1741; Fax: 706-542-5865
- Wildlife Health Center, University of California, TB128, Old Davis Road, Davis, CA 95616. Tel: 530-752-4167.

CANADA

- Canadian Cooperative Wildlife Health Centre, WCVM Vet Pathology, University of Saskatchewan, 52 Campus Drive, Saskatoon, SK S7N 5B4. Tel: 306-966-5099, Fax: 306-966-7439.

Enhancing the skills of the biologist in wildlife disease investigation is the primary goal of this chapter. We realize that many techniques in this chapter are described as if implemented under ideal conditions frequently not encountered by biologists. The exact tools, containers, preservatives, etc. may not be available. However, in most circumstances field samples collected under less than ideal conditions are better than none at all and can provide important clues to the cause of the mortality event.

Before embarking on investigation of wildlife mortality events, it is important to recognize that some diseases of wildlife are zoonotic (transmissible between, and capable of causing diseases in, animals and humans). These diseases can be transmitted through aerosols (inhalation), ingestion (including food or hand-mouth contact), or contact through mucus membranes (eyes, mouth), cuts on the skin, or insect bites. While risk of this happening is most significant with mammals because of closer similarity to humans, birds, reptiles, fish, and invertebrates can also, at times, carry pathogens transmissible to humans. Another consideration is the potential for toxins that can pose a risk to investigators. Investigation of wildlife diseases, while

potentially rewarding from a management standpoint, can be risky.

To limit potential exposure to pathogens and toxins, biologists and disease specialists must implement appropriate protective measures including involvement of qualified medical personnel in decisions on personnel vaccination, if warranted. At a minimum, when handing dead animals or animal tissues, personnel should wear protective clothing, and manipulation of specimens should be done with gloves that are impervious to body fluids. Biologists should be knowledgeable about the zoonotic diseases in their area. This information can be obtained from the local public health department. Many zoonotic diseases have nonspecific lingering "flu-like" symptoms, and some, if left untreated, can have serious long-term complications. Most physicians have little knowledge of zoonotic disease. Therefore, if the biologist suspects they have contacted a zoonotic disease, they may need to participate in education of their physician, impress upon them the possibility of a zoonotic disease, and remain closely involved in medical management of their case to ensure successful resolution of the illness.

DISEASES OF WILDLIFE

Three factors need to be present for disease to occur in wildlife populations: the agent, the host, and the environment. These must interact in a manner that disease can be expressed. The agent must be *capable* of producing disease in the host. For example, rabies virus is not capable of producing gastroenteritis but is capable of producing encephalitis in mammals. Some agents are obligate pathogens—that is, under appropriate circumstances, and for propagation of the disease agent, expression of disease is required. Other agents may be opportunistic. An opportunist may or may not produce disease depending on host or environmental characteristics. The host must be *susceptible* to that agent. For example, a mammal would generally be susceptible to rabies but a vaccinated individual may mount an effective immune response preventing disease. Only birds in the Order Anseriformes are susceptible to the viral agent that causes duck plague. An animal with nutritional insufficiency or deficiency may be susceptible to many disease agents to which it otherwise would not be susceptible. Lastly, a *suitable* environment for disease expression must be present. For example, with the disease brucellosis the suitable environment is one in which the bacteria can survive in an aborted fetus for sufficient time for another animal to ingest an infective dose. Sunlight and drying may rapidly inactivate an agent. If environmental conditions prevent contact of another animal with an abortion (unsuitable environment), the disease does not perpetuate.

Several basic terms are used to describe disease characteristics in populations.

Epidemic/Epizootic. This term describes a level of disease in populations that is above "normal" expected baseline values (see "endemic"). An example of epidemic disease is the occurrence of large die-offs of waterfowl in wildlife refuges. However, a single case of a foreign animal disease (one not found historically in a particular location) can be considered an epidemic.

Endemic/Enzootic. This describes a disease that is present in an animal community over the long term and frequently refers to the "usual" frequency of disease in a population or the constant presence of disease in a population. Presence of rabies in some populations of wild carnivores is a good example.

Prevalence. This is the number of *existing* cases of a disease in a known population (or sample size) at a specific time. Prevalence calculations are static indicators and are used to describe the existing status of disease in populations.

Incidence. This is the number of *new* cases of a disease in a known population over a specified period of time. Incidence calculations are dynamic indicators and are used to describe the progression of disease in a population over time.

Wildlife diseases are most notable when they cause catastrophic mortalities of large numbers of animals over a short period of time. These large mortality episodes may have a detrimental impact on wildlife populations and can have grave consequences when the species affected are endangered or threatened. An example of such a disease is avian cholera; a bacterial disease that is rapidly disseminated from bird to bird and that can cause large mortalities of waterfowl in wetlands. Avian malaria, a protozoan-caused disease transmitted by mosquitoes, has been associated with extensive mortalities of endangered Hawaiian forest birds and has been implicated in the disappearance of several species of native birds. Other wildlife diseases are more chronic and insidious. These diseases may affect demographic factors such as reproductive success or recruitment of young. An example of this is marine turtle fibropapillomatosis, a tumor disease that causes progressive wasting of predominantly immature green turtles (*Chelonia mydas*). Noninfectious agents can also cause significant disease in wildlife. Severe acute mortalities of birds and mammals have been traced to ingestion of toxins produced by fungi present in moldy grain. Lead poisoning from ingested lead shot was a significant cause of chronic mortalities in endangered bald eagles (*Haliaeetus leucocephalus*) and waterfowl until lead shot was banned for use in hunting migratory waterfowl in the United States.

Investigation of wildlife diseases is largely a deductive and iterative process. When confronted with wildlife mortality, the investigators (biologists and disease specialists) must initially consider a wide variety of possible causes both infectious (e.g., viruses, bacteria, parasites, fungi) and noninfectious (e.g., trauma, toxicants). Using a combination of field and laboratory observations, the investigators systematically eliminate the less plausible causes of the mortality and eventually arrive at a subset of the most likely cause(s). In many cases, the investigation will not point to a single cause; this is often a point of frustration for the biologist. However, in most cases, it is just as valuable to know for certain what did ***not*** cause the mortality as this information alone can help managers focus on management options. There are many potential causes to consider at the outset of the investigation and an investigator must keep an open mind as to the possibilities. An easy to remember acronym that captures most causes of disease is "DAMN IT." Specifically: D (degenerative and developmental conditions), A (allergic and autoimmune conditions), M (metabolic conditions), N (neoplastic [cancer] and nutritional conditions), I (infectious and idiopathic [unknown] conditions), and T (traumatic and toxic condi-

tions). Interactions among these classes of disease may also occur, such as toxic insults resulting in neoplasia, or immune deficiency allowing an opportunistic infectious agent to cause disease.

While wildlife can be affected by all the above etiologies, those most commonly encountered fall into the nutrition, idiopathic, toxic, infectious, traumatic, and sometimes neoplastic categories. Some diseases will affect particular age groups of animals. For example, it is common to see large numbers of immature seabirds die from starvation shortly before or during fledging because a certain percent were unable to forage adequately or were abandoned by inexperienced parents. Brucellosis, a bacterial disease of ungulates, affects principally females that manifest disease through abortions or infertility. Certain diseases are more likely to occur in certain habitats. Wetlands are common areas where diseases of waterfowl such as botulism or duck plague are encountered. Other diseases depend on presence of insects for transmission and spread, and are absent where the vectors are absent. The absence of the insect vector for bluetongue virus explains the absence of this disease in deer in certain areas of the United States.

It is the basic understanding of these "epidemiological" relationships that helps narrow the list of possible causes and guides laboratory testing. The science of epidemiology is the study of the elements that contribute to occurrence or nonoccurrence of disease in a population. The wildlife disease specialist uses knowledge of these relationships to avoid unnecessary, sometimes expensive, testing and to guide further field investigation.

Investigation of chronic wildlife disease proceeds in a similar manner as acute diseases; however, there is more dependence on longer-term observations. Surveys of wildlife populations for pathogens involve capturing animals and taking biological samples including blood, feces, urine, and biopsies, or doing necropsies of dead animals over long time periods. Marking animals and following presence of disease over time can be valuable to clarify complex disease patterns. Use of geographic information systems and mathematical models are also becoming important in studying behavior of disease in wildlife populations and offering management options.

TOOLS AND METHODS TO INVESTIGATE WILDLIFE DISEASE

Field Observations

Careful recording of field observations and conditions is the first, most important tool in addressing wildlife disease and mortality. The clues provided by a detailed and accurate history of the event can narrow the possibilities for diagnosis. Some of the parameters associated with the disease outbreak that should be recorded are below.

Magnitude: is the morbidity/mortality unusual? How many?
Onset: when and how rapidly did the situation develop?
Environment: are there unusual or novel circumstances? Habitat types? Human uses and what type? Nearby land uses? Spatial distribution of mortality?
Species affected and species at risk: what species are dying/sick compared to what species appear to be at risk for the disease?
Population at risk: what is the size of the population that is affected?
Population movement: where did the animals come from and where do they go?
Age and gender: do affected animals tend to be an age or gender specific?
Mortality and morbidity: are both dead and sick animals observed? What is the approximate ratio of sick to dead?
Clinical signs: if sick animals are seen, what physical or behavior abnormalities are apparent?

Documenting field observations is critical and can be done using write-in-the-rain notebooks and pencils to palm and laptop computers. The critical step is to carefully annotate events and their temporal sequence. Photographs can be useful in wildlife disease investigations. The advent of digital cameras has made it possible to instantly send photographs anywhere in the world for immediate consultations. However, photos must suitably depict the subject. Most importantly the subject should be recorded with sufficient image size and depth of field for identification of what is being viewed. Thus, a long distance view of a sick bird might provide information on the environment affected, but a close-up will be needed to assess clinical signs. When photographing lesions during field necropsies, the organ with the lesion rather than the entire carcass should predominantly occupy the field of view. It is sometimes helpful to take several exposures that vary in distance from the subject. Photos should be labeled so they can refer to specific animals/specimens. Digital videos of sick animals can also provide valuable information and complement field descriptions of clinical signs.

Magnitude and Onset

One of the most difficult issues is when to call a wildlife mortality event "unusual" and when to assign the time of onset. Unless the population is closely monitored and the biologist understands the background mortality in that population, assigning time of onset and whether the mortality is unusual is problematic. Many wildlife species, when ill, seek cryptic locations complicating the discovery of carcasses. Regular (monthly) patrols and cataloguing mortalities by location, species affected, and estimated status of decomposition provide valuable background information that can be used to make decisions as to whether or not mortality is unusual. Developing knowledge about normal behaviors and movements of wildlife facilitates detection of sick or abnormal animals. In reality, true onset of wildlife mortalities is seldom detected.

Environment

Establishing potential links between changes in the physical environment and wildlife mortality is important. If disease is occurring where previously there was none, something has changed in the environment. Many possibilities with regard to animal population and environmental changes should be considered.

Temporal Distribution

Assessing the temporal scale of the wildlife mortality can provide critical information on the potential cause(s)

of the event. Spills of toxicants or natural toxins may kill large numbers of animals over short periods of time (days to weeks). Examples include avian botulism or organophosphate insecticide mortality. Certain infectious diseases, particularly when introduced where animals are concentrated, may kill many animals over several weeks. Mortalities diminish when immune animals remain, when animals emigrate from the area of disease transmission, or the source of the infectious agent is no longer available. An example is mortality due to acute aspergillosis that ceases once the grain pile containing high amounts of the fungus is no longer available. Other diseases cause losses over longer periods of time (months), and mortalities can increase and decrease depending on demographic and environmental factors. For example, brucellosis occurs in a narrow part of the reproductive cycle, and primarily in first pregnancy post-exposure animals. Losses to the population thus only occur at certain times of year, in only certain populations, and at varying rates depending on exposure history. Phocine distemper causes periodic mortalities in seals (Phocidae) in the eastern United States and Europe; seals tend to be particularly prone to disease when concentrated. Some diseases are only detectable when animals congregate in particular areas. For example, acute lead poisoning is most evident when waterfowl are staging for migration in wetlands contaminated with lead shot. Consequently mortalities due to ingestion are most likely to be detected when large numbers of animals are exposed. In contrast, chronic lead poisoning can be inapparent as slowly debilitating animals seek refuge before death, are killed by predators or disperse to other areas.

Species, Age, and Gender

Species, age, and gender of animals affected can also provide valuable indicators as to what may or may not be causing mortality. Starvation tends to disproportionately affect young or old animals. Some species are more sensitive to certain diseases than others. For example, northern shovelers (*Anas clypeata*) tend to be highly efficient sifters of sediments for invertebrates. Because botulism toxin is concentrated in invertebrates, shovelers are one of the first animals to be affected during botulism outbreaks. Other causes of wildlife mortality, such as pesticides, are more indiscriminate and will affect several species. Raptors, for example, can die from consuming smaller birds that die from organophosphate poisoning. Younger animal have a less developed immune system or different behaviors than adults and can be more susceptible or receive higher exposure to certain infectious agents; thus, they may be overrepresented during certain disease outbreaks. Other diseases may affect one gender preferentially either because of behavioral or physiologic differences. Avian cholera affects female common eiders (*Somateria mollissima*) more than males because, during disease outbreaks on nesting grounds, males are at sea (Korschgen et al. 1978). Brucellosis, a pathogen of the reproductive tract in mammals, tends to affect females more severely and manifests as abortions.

Clinical Signs

Clinical signs of sick animals should be carefully documented and, if possible, videotaped. Animals with poor hair or feather appearance with large burdens of ectoparasites are clearly abnormal. Many diseases cause neurological abnormalities such as inability to turn right side up, tremors, improper posture of limbs, abnormal gait or flight, or inability to keep the head upright. Clinical signs can also be characteristic. For example, brown pelicans (*Pelecanus occidentalis*) affected by the marine toxin domoic acid exhibit scratching behavior alternating between the left and right legs. Other diseases induce changes in temperament. Rabies will often make normally wary wild carnivores more docile or aggressive. At times, sick animals will be in unusual locations or apart from others. Absence of sick animals during wildlife mortality episodes can be just as an important clue as presence.

Geographic Distribution

Potential causes of wildlife disease can be gleaned from spatial patterns of wildlife mortalities. Carcasses in one particular area could suggest they were concentrated there by wind or current, or there was a point source of mortality in that area. Presence of severely decomposed carcasses along with fresher specimens would indicate mortality has been ongoing. Daily movement patterns of animal populations should be recorded as well as location of common foraging and loafing areas. Particular notation should be made of movement from a mortality area to other areas as this can help investigators map disease spread and take pre-emptive management actions. This information can help managers identify the source of disease outbreaks or alter the course of disease at locations to which wildlife may move. Knowing population movement patterns also affects timing and feasibility of intervention strategies. For example, in an attempt to eliminate chronic wasting disease from the first site of discovery on the western slope of the Rocky Mountains in Colorado, managers had a limited window of opportunity in which to take action before spring migration dispersed cervids to higher, inaccessible areas. Changes in ratios of animals affected (gender, age, or species) can provide valuable clues to wildlife disease specialists. At a minimum, location of carcasses should be plotted on a map. These locations should be by date of observation so that spread of the epizootic can be followed. Global position satellite technologies now allow users to add information about location including weather, diagnostic findings, etc. that can be later used to evaluate characteristics such as movement, distribution, and incidence of disease in the population.

Laboratory

Detailed field observations provide an invaluable starting point to initiate disease investigations. Proper collection and submittal of samples to appropriate laboratories is the next logical step. For many biologists, this process is a "black box" from which emerge results that are often presented in arcane language and format with little apparent management value. Poor communications between laboratory and field can easily derail the disease investigation and make management ineffective. This section is designed to aid biologists in their understanding of the basic principles used in laboratory investigations. Our intent is to help biologists understand the limitations, advantages, and requirements of each discipline. This knowledge will help biologists discuss laboratory results with wildlife disease specialists, collect the most useful

specimens, and make better decisions based on laboratory findings for assessing and managing the field situation. These laboratory tools are designed to identify infectious and noninfectious agents that could be potential causes of observed mortalities.

Pathology

Wildlife disease investigations in the laboratory typically begin with an examination of dead animals. In pathology, a complete external and internal exam of the carcass (gross necropsy, postmortem or autopsy – generally autopsy is used in conjunction with human postmortems) is conducted and samples of animal tissues are preserved for further examination under the microscope (histopathology) or for tests in other laboratories. During the external exam, the animal is weighed and measured, and the presence of any evident abnormalities (emaciation, skin abnormalities, etc.) is recorded. All major internal organs are examined and changes in their color, consistency, appearance, number, distribution, and size are noted. Pathology and histopathology can only detect morphologic change in organ systems, organs, tissues, and cells. Biochemical, submicroscopic, and other nonanatomic changes cannot be visualized and detected by pathology. Electron microscopy can enhance detection of microorganisms such as viruses, but the technique is limited to having a sample with the offending organism present.

Tissue samples for histopathology are stored in 10% buffered neutral formalin (a mix of salts and formaldehyde) and formaldehyde or ethanol. Tissues from a single animal can be stored in one jar, the size of the tissue fragments should be sufficiently thin (6-8 mm) and the volume of formalin sufficient (at least 10 × the volume of formalin to tissues) to allow adequate penetration and fixation of tissues. Tissue should be collected with a sharp knife or scalpel and crushing the tissue when handling with forceps should be avoided. If a lesion is seen, it is critical the tissue section contain both tissue with the lesion and adjacent unaffected tissue because many pathologic diagnoses are based on observation of the transition between normal and abnormal tissue. Normal lung tissue will float in formalin and it is important to note if they sink. Tissue sections should not be so large as to curl during fixation. Only wide mouth containers should be used because tissues that easily slide into containers when fresh can be difficult to remove once fixed if the mouth is narrow. Tissues fixed in formalin should be stored at room temperature and kept from freezing. Inadvertent freezing, such as leaving the samples in a truck overnight during winter, can ruin samples for histopathology.

Samples are later sent to the laboratory where they are embedded in paraffin, trimmed into thin slices, placed on microscope slides, stained, and examined under the microscope by a pathologist who looks for abnormalities at the cell and tissue level. The findings may indicate the need for specialized techniques, such as use of stains that increase the visibility of bacteria, fungi, or internal parasites. Pathology can provide a wealth of information on what did or did not cause death in an animal and the results can be used to focus additional laboratory and field investigations.

The main limitation of pathology is condition of tissues. Decomposition, freezing, or improper fixation damages tissues and cells. The more damage by these postmortem factors, the less the pathologist can accurately interpret as the effect of disease. Severely altered tissue will allow a pathologist to find only the most obvious, but not necessarily the most significant, disease-caused changes. Biologists have a critical role in ensuring that specimens (carcasses) collected for pathology are as fresh as possible. The ideal specimens for pathologic exam are fresh, chilled unopened carcasses, individually wrapped and submitted to the diagnostic laboratory. A rule of thumb is to send fresh specimens if they can be rapidly chilled after discovery and delivered to the diagnostic laboratory within 24 hours (48 if the timing of death is known to be in the past 6 hours). If this is not possible, frozen carcasses can be submitted although freezing limits pathologic interpretation. When the carcass is large or when the location is remote, the biologist must do the gross examination, take careful notes of findings, and preserve tissues in formalin. Because gross pathology is an inherently low-technology tool, it lends itself to remote field situations. With practice, field biologists can become adept at recognizing normal from abnormal during gross exams. Necropsy kits can be assembled at minimal costs; tissues can be stored in formalin at room temperature and later sent to the laboratory. Several resources exist explaining the details of how to conduct necropsies and collect and preserve tissues of ungulates and birds (Roffe et al. 1994, Friend and Franson 1999), sea turtles (Rainey 1981), fish (Meyer and Barclay 1990), and marine mammals (Dierauf and Gage 1990).

Bacteriology/Mycology

In these disciplines, tissues, fluids or swabs from tissues taken during necropsy are processed in the laboratory to culture bacteria or fungi that may be responsible for the mortality. Bacteriology/mycology can document the presence of living microorganisms, assay samples for evidence that the nucleic acid of a suspected pathogen is present (e.g., through polymerase chain reactions) or assay for biotoxins produced by these microorganisms. If the submitted sample does not contain the causative agent, its toxin, or the causative agent/toxin has degraded; the laboratory will provide a negative finding. A living microorganism or intact toxin must be present in the sample for the usual techniques to be effective. A negative finding may lead to an erroneous conclusion that a particular bacteria or fungus was not responsible for the mortality. Interpretation of the findings has to be made in view of field events and known sampling limitations.

Typically, tissues for bacteriology are collected using sterile techniques and placed individually in sterile plastic bags. Alternatively, sterile swabs can be inserted in the tissues and fluids can be aspirated or swabbed. Swabs or tissues should be refrigerated (not frozen) and sent to the laboratory as soon as possible. However, many bacteria, fungi and their toxins survive freezing quite well. These samples are better than decomposed specimens. In the laboratory, tissues or swabs are placed in special media that encourage growth of bacteria or fungi. Media used are chosen based on their ability to selectively grow certain microorganisms and inhibit growth of others. Once bacteria or fungi are cultured in the laboratory, those thought to be significant are identified with a battery of biochemical tests. Repeated sampling may be needed during an outbreak to confirm the

original agent is still responsible for continued mortality or to track the pathogen into other populations. This information allows managers to maintain or alter management response for optimum effect.

The main limitation of bacteriology/mycology is the condition of specimens relative to postmortem decomposition and preservation. A variety of opportunistic microorganisms will rapidly colonize a carcass and the number and variety of these contaminant organisms increase with time of decomposition. Colonization by contaminant organisms in tissues can hinder laboratory interpretation of results, mask the presence of the original pathogen, or kill the pathogen. Thus, it is critical that samples for bacteriology be taken using sterile techniques and immediately shipped to the laboratory. Poor sampling techniques or leaving samples refrigerated for long periods of time (days) prior to culture in the laboratory increases chances for secondary contamination.

Virology

Virology is similar to bacteriology in that tissues are processed in the laboratory in attempts to grow viruses from tissues or detect fragments of their nucleic acids. Typically, tissue samples are collected using sterile techniques and placed individually in labeled, sealed plastic bags where they are stored frozen (–70 C optimal). In the laboratory, tissues are homogenized in special media and placed in cell cultures. Because viruses use and kill animal cells to replicate, their effect can be detected in the culture. Laboratories may also use special staining techniques directly on samples for examination under the electron microscope to look for viruses. Additional molecular and immunologic tests are used to specifically identify the virus. As with bacteriology, virology will provide a positive response only if the sample contains virus particles that can be detected by the method of choice. Inactivated ("dead") viruses will not be detected on cell cultures and the result will be negative. False negative findings are even more common in virology than bacteriology because of the more fragile nature of viruses, greater susceptibility to handling errors, and because many viruses disappear after causing initial organ damage.

Virology requires that tissues be freshly refrigerated or rapidly frozen to ensure viruses are properly preserved in the sample. When freezing tissues, it is critical that tissues remain frozen until they arrive at the laboratory (however, the carbon dioxide produced by dry ice can inactivate some viruses). Viable viruses are less likely to be found in tissues that are decomposed or that have been repeatedly thawed and frozen. Unfortunately, because virology is expensive and requires particular expertise, relatively few laboratories are equipped to do these assays. Thus, virology is usually attempted only if there is evidence (based on gross and microscopic pathology) that viruses may be responsible for the animal mortality.

Parasitology

Parasitology is the study of organisms such as worms, protozoa, mites, and ticks. Some parasites can be visible to the naked eye and others can only be seen using microscopes. Parasites frequently occur in wildlife and may be a cause of mortality. If parasites are the causative agent for wildlife morbidity/mortality, they occur in large numbers and with associated tissue changes. Knowledge of "normal" parasite loads is important to interpreting findings. The mere presence of worms in lungs or intestine does not mean that organ was significantly compromised. As with other diagnostic disciplines, positive findings are conclusive but negative findings can be false negatives. False negative results are less common in parasitology than other disciplines, and considerably less likely to happen for metazoan (multicellular) parasites.

Some macroparasites (round worms, tapeworms, lice, fleas, and ticks) are preserved in labeled vials containing 70% ethanol. Other parasites (spiny-headed worms, flukes) are best preserved in alcohol-formalin-acetic acid. Parasites preserved in this way should be stored at room temperature and submitted to specialists for identification. Detection of microparasites such as blood parasites or skin mites requires examination of blood smears or skin scrapings under a microscope. A variety of specialized techniques are used to culture and identify protozoa, or to detect larval stages of worms or parasite eggs (Box 2).

Box 2. Worm egg flotation.

Numerous methods exist to detect parasites, but fecal flotation for nematode (round worms) eggs is the most common. This technique relies on thoroughly mixing fecal samples in a liquid with specific gravity higher than that of the eggs. Eggs then float to the top and can be collected. Nematode eggs have a specific gravity just slightly higher than water; thus a liquid with a specific gravity of 1.1–1.2 is used. In contrast, trematode (fluke) eggs have a much higher specific gravity and this technique will not detect these parasites.

Materials needed

- fecal sample
- flotation solution (generally saturated salt, sugar or magnesium sulfate)
- test tube and holder
- slides and cover slips
- stirring instrument (stick)
- compound microscope

Procedure

1. Place test tube in rack and fill the bottom one-third with feces.
2. Fill the remainder of the test tube with flotation solution leaving sufficient room for stirring.
3. Using the wooden stirring stick, thoroughly mix the solution and feces.
4. Immediately top off the test tube with flotation solution sufficient to bulge slightly above the rim.
5. Place cover slip on top of test tube.
6. Allow to sit for one half-hour. Eggs will float to the top and stick to the cover slip.
7. Gently lift cover slip, place on glass slide and examine under the microscope.

Toxicology/Toxinology

Toxicology uses a variety of techniques to identify the presence of particular chemicals in animal tissues or fluids. Toxicology refers specifically to the detection of man-made chemicals (e.g., insecticides, and rodenticides) while toxinology refers to detection of natural poisons (e.g., botulinum toxin or marine toxins). Laboratory assays for chemicals are complex and require expensive equipment. There are specific requirements to sample and package tissues for submittal to the laboratory. For example, tissues to be analyzed for heavy metals (e.g., lead, cadmium) are typically stored frozen in sterile plastic bags and should not contact metal objects. Wrapping the tissue in aluminum foil is desirable for certain other chemicals prior to freezing. Some chemicals are more resistant to decomposition than others, and some are more likely to be detected in one type of tissue or organ. There may be additional requirements to procure environmental samples (water, soil, and food) to trace the source of the chemical in the environment (Sheffield et al. 2005).

Toxicology results can be relied on more when a negative finding is reported. However, tests for some toxins (e.g., botulinum type E) are frequently negative even when they are the actual cause of mortality. The behavior of many toxins in biological samples and the environment is well characterized so a reasonable prediction can be made about the reliability of negative findings. The ability to detect a positive sample frequently depends on the sensitivity of the assay used. Generalized techniques (or assays) for toxicants are uncommon. Thus, questions must be more specific and assays must be for specific toxins. Because of the expense of testing, analyses for chemical toxins are rarely done before a thorough field investigation and postmortem examination suggest a particular toxin may be involved (Sheffield et al. 2005). The best approach for collection of tissues for toxicology is to have preliminary information suggesting the possibility of a specific toxin. The laboratory used for testing should be consulted for the best method of sample collection, handling, and shipping.

Molecular Biology

Use of the polymerase chain reaction (PCR) has revolutionized the detection of microorganisms and augments standard bacteriology, virology, and parasitology techniques. Polymerase chain reaction is a laboratory technique for amplification of DNA. Amplification permits detection of DNA that otherwise would be missed. For example, a single strand of DNA from a virus in a tissue sample can be amplified by 1,000. This powerful technique has enhanced the sensitivity of many assays for a wide variety of micro-organisms and an increasing number of laboratories have or are establishing capabilities to do PCR. Tissues for this assay can be individually wrapped in labeled plastic bags and stored frozen, preferably at –70 C.

The limitations of PCR are quite different from other techniques. First, the technique detects only genetic material and not viable organisms. Hence, the presence of DNA from a virus in a tissue sample does not necessarily indicate the virus was viable and replicating or even causing disease. Second, because the technique depends on the amplification of known sequences of DNA, an investigator must decide which virus or bacteria to seek. Third, if samples are contaminated with genetic material not originally found in the specimen of interest, a false positive finding may occur. Contamination can occur through specimen handling, instruments used for specimen collection, and packaging of multiple specimens, among other ways for cross-contaminating specimens. However, amplification and detection of a known genetic sequence is confirmation that the suspect organism was present at some time in the host's environment. Polymerase chain reaction technology is best used once field investigation and pathology have suggested the likelihood of a particular microorganism or if there is a high need to eliminate or confirm the presence of a specific pathogen. This technology is generally used to confirm the identity of pathogens that have been detected by other methods.

Serology

When microorganisms infect animals, the animals usually develop an antibody response towards that organism. The ability to generate antibody and the level of antibody production is host-agent specific. Serology is the technique used to detect and measure levels of antibodies in serum. Blood is collected (Fig. 1) from animals and centrifuged to separate cells from liquid. It is important not to freeze the blood before centrifugation. The cell-free liquid portion of the blood (serum) is removed and stored frozen in labeled vials for later analysis for antibodies. A variety of serologic assays exist to detect antibodies. Some can be used directly in the field while others are more complicated and must be done in specialized laboratories. Many assays are developed specifically for use in domestic livestock or small animals (dogs and cats) but are often used in wildlife species. However, validation of techniques developed in domestic animals for wildlife is frequently lacking.

Serologic assays detect exposure to an infectious agent (or similar micro-organism) but do not detect presence of disease. This is an important distinction because it is often assumed that presence of antibodies to an infectious agent indicates that disease from this organism is present. Most "serosurvey" results only reflect historical exposure and provide no evidence of disease or disease impact on the population. However, properly used serology can infer presence or absence of particular microorganisms, and inferences regarding disease can be made. As examples, brucellosis antibodies have been shown to be positively correlated with presence of the organism in bison (*Bison*

Fig. 1. Blood sample collection from a duck using jugular venipuncture and vacutainer system (photograph courtesy of T. J. Roffe).

bison) and elk (*Cervus elaphus*) (Roffe et al. 1999). Higher antibody levels correspond with a higher likelihood of being infected; high levels are often a good predictor of impending abortion. In comparison, low levels of parainfluenza antibodies are frequently found in ungulate populations and are probably meaningless relative to population effects. In individual animals, a 4-fold rise in titer of specific antibodies using paired serum samples is considered a reasonable indication the specific agent is present and replicating at the time of mortality or morbidity.

The timing of the rise in antibodies relative to disease and specificity of the test for antibodies are critical to make this inference about a disease agent. Whether that agent can be implicated as the cause of mortality or morbidity also depends on whether the field investigation and pathology corroborate the effects that agent would be expected to produce. Many serological tests developed for domestic animals use strains of pathogens or reagents specific for that animal. When used in wildlife species that are often infected with different strains of the organism, these tests can give misleading results (false positives and false negatives) or can lead to confusion as to what exactly constitutes a positive or negative result. In addition, some serologic tests measure antibody to the microorganism of concern and closely related microorganisms. Thus, knowledge of the specificity of the test is essential for interpretation. Serological tests can prove useful to trace the presence of a microorganism in animal populations.

Clinical Chemistry and Hematology

Clinical chemistry is the analysis of serum or plasma for proteins, enzymes, metabolites, and minerals. Whole blood is collected and the serum or plasma is separated, stored frozen, and subsequently chemically analyzed using automated analyzers. Damage to or improper function of internal organs (e.g., liver, kidney, pancreas, and muscle) will lead to changes in levels of specific proteins, enzymes, and minerals in the blood. The change may be an increase or decrease in a particular serum constituent depending on which organ is affected; however, interpretation is difficult because normal values for many species are unknown. Elevated levels of a particular enzyme might suggest liver disease while elevated levels of a particular mineral could suggest kidney disease. More frequently, the combination of elevations and decreases in multiple parameters may suggest a disease entity. Clinical chemistry is routinely used by clinicians to obtain an idea of how internal organs are functioning in living animals but is not useful for dead animals because of changes in parameters associated with decomposition.

Hematology is analysis of whole blood for quantification and morphologic evaluation of cellular components and, like clinical chemistry, is only useful in living animals. Whole blood collected in anticoagulants is necessary for cell counts. A drop of blood is placed on a microscope slide and stained to permit enumeration of the different types of cells or detection of blood parasites. The percentage of whole blood that is red cells (hematocrit) can be measured through centrifugation. Other assays can be used to detect hemoglobin content and morphology of red cells. Automated hematology analyzers are available for domestic animals. Some inflammatory processes will lead to elevation of specific types of white blood cells or decreased numbers of red cells; this can be detected through hematology.

Hematology and clinical chemistries can be useful to assist disease investigators in assessing which organ systems are likely affected by the disease agent, but they rarely provide a specific diagnosis. The goal with these tools is to narrow the list of suspect agents by detecting changes in the animal's physiology that are consistent with a limited number of agents. These tests require blood samples taken by trained personnel and generally require live, captured animals. Some hematology tests must be conducted shortly after blood collection making them impractical to use in remote field settings.

Most laboratories operate on and provide normal values for domestic animals. They do not have the expertise to assess hematology findings from wildlife species. Thus, interpretation of results must be done with care and in consultation with appropriate experts.

Epidemiology/Epizootiology

These terms are frequently interchanged in regard to animal populations. Epizootiology originated in reference to the science of epidemiology as applied to animals. Dictionary definitions of epidemiology refer to human communities. However, the origin of the term epidemiology is derived from the Greek for "study of something prevalent" and is equally applied to people and other animals. Epidemiology is the study of the relationship of factors affecting the frequency and distribution of disease in animal populations. A good general reference for the biologist is Thrusfield (1997). Epidemiology brings together laboratory findings and field observation using mathematical/statistical methods to detect trends and patterns in diseases of wildlife populations. At its most basic, epidemiology is the summarization of percent of animals affected by a particular agent. Or it may be complex, involving elaboration of mathematical models and spatial analysis to explain or predict behavior of disease in animal populations.

FIELD NECROPSY TECHNIQUES

When it is not possible to submit carcasses directly to a diagnostic laboratory, it will be necessary for the biologist to conduct necropsies in the field. Wildlife biologists contemplating doing so should obtain formal instruction from trained individuals. A variety of institutions provide guidance and training on necropsy procedures and sampling. A few institutions (e.g., National Wildlife Health Center, U.S. Geological Survey; Southeast Cooperative Wildlife Disease Study, University of Georgia) have specific training programs for teaching biologists about wildlife disease investigation techniques and necropsy procedures. While variations exist on the anatomy of different species of animals, the principles of necropsy remain the same. The first step is obtaining a detailed history of the circumstances surrounding the death of the animal. Thus, the animal should be observed *in-situ* for clues in the environment that could help explain the mortality.

Once this information is noted, the carcass can be moved to a suitable location (Fig. 2). The area where the necropsy is done should be as comfortable as possible,

Fig. 2. Using heavy equipment to transport a dead bison before necropsy (photograph courtesy of T. J. Roffe).

Fig. 3. Modified pit burning disposal of waterfowl carcasses. Use of a grate allows for more rapid and complete burning (photograph courtesy of T. J. Roffe).

well ventilated, and should have access to running water. Tools appropriate to the carcass to be examined should be available (Table 1). If the carcass cannot be moved to a suitable location, the necropsy must be done on site. However, it is important to check with local authorities to ensure compliance with any ordinances, to warn them of possible complications, and/or to make arrangements for proper disposal of remains (Fig. 3). Assuming the carcass is small enough to permit relocation, efforts should be made to prevent contamination of the area with body fluids. The best facility is one designed for necropsy with a drain and stainless steel tables. On other surfaces plastic sheets and tarps can provide a barrier against contamination.

Table 1. Components of a field necropsy kit.

Essential items	No.	Other useful items	No.
Scalpel handles	3	Bone snips	1
Large scissors	2	Hemostat	1
Toothed forceps	3	18G 1 ½" needles	100
Shears	1	20G 1 ½" needles	100
Scalpel blades	12 dz	22G 1 ½" needles	100
Sm whirl-pack bags	150	5-cc syringe	50
Various plastic bags	100	10-cc syringe	50
Ties for plastic bags	100	35-cc syringe	5
Bottles with 10% buffered Formalin	2	Instrument tray with cover	1
Plastic bottle undiluted Roccal	1	Microscope slides	1 gr
Waterproof markers	3	Coverslips	1 bx
Pens	3	Face masks	100
Pencils	3	Clipboard	1
Necropsy sheets	100	Dissecting pans	2
Specimen bottles	2	Zip-loc bags	100
Dry ice shipping labels and instructions	2	Paper towels	100
Nondisposable gloves	6 pr	Large whirl-pack bags	100
Disposable gloves	6 pr	Labels	1 gr
Ruler	1	Clear tape	1 roll
Strapping tape	2 rolls	Ringing sticks	1 gr
Aluminum foil	1 roll	Test tube racks	2
Straight butcher knife	1	Centrifuge tubes	100
Curved butcher knife	1	Gauze	1 roll
Blue ice packs	10	Slide boxes	2
		Vials with screw caps	144
		Swabs	100
		Hatchet	1
		Alcohol lamp	1
		Lighter	1
		Bottle fuel for lamp	1
		Boot brush	1
		Label tape	2 rolls
		Bone saw	1

Prior to opening the carcass, it should be weighed, if possible, and an external exam completed. The key element to a postmortem exam is proceeding in a systematic manner (head to tail or vice versa) so that details are not overlooked. Mouth, nose, and eyes should be evaluated for discharge (blood, froth, and excessive mucus). Limbs should be palpated for broken bones (palpating both limbs on each side simultaneously helps in detecting abnormalities). Hair/feather/scale coat should be examined for abrasions, trauma, or ectoparasites. If no abnormalities are seen, this should be noted because absence of written records can imply the observation was not done.

The internal exam must be done in a systematic manner (Box 3) with all organs examined in a consistent order. Usually it is best to leave examination of the gastrointestinal tract until the end of the necropsy to minimize contamination of other organs with ingesta (and gut bacterial flora). During the internal exam, abnormalities in color, shape, size, texture, and number of organs should be noted. Recognizing normal from abnormal is challenging for the beginner and an appreciation of the difference can only be gained through practice and examination of many animals. If in doubt, it is better to record what appears to be abnormal than to record nothing at all. Photographs of suspected lesions can be electronically sent to the pathologist or enclosed with the gross necropsy report. More detail is better than less detail in the necropsy report, as this will aid the pathologist during the microscopic examination of tissues.

Thorough decontamination of the necropsy area and tools should be done after the necropsy to minimize risks of disease transmission. Scrubbing the area and tools with soap and water followed by rinse with 10% bleach or commercial disinfectant is usually sufficient to eliminate infectious organisms.

Box 3. General internal postmortem exam procedures.

1. Ungulates, small carnivores, and other laterally compressed species.
   a. Place in left lateral recumbency (on left side, places rumen down).
   b. By cutting with a knife, reflect right fore and hind legs over body.
   c. Make midline skin incision from tip of lower jaw to pubis.
   d. Reflect right side of skin over the back exposing all muscle and subcutis from head and face to pubis.
   e. Reflect penis or mammary gland downward, toward the left rear leg.
   f. Open small window into abdomen just behind last right rib by cutting through the abdominal wall. View internally for abnormal fluid, blood or exudate.
   g. Extend this window by cutting away the right lateral abdomen wall as a flap that remains attached along the midline. Make the window large enough to view all organs but not so large as to allow viscera to spill (Fig. 4).

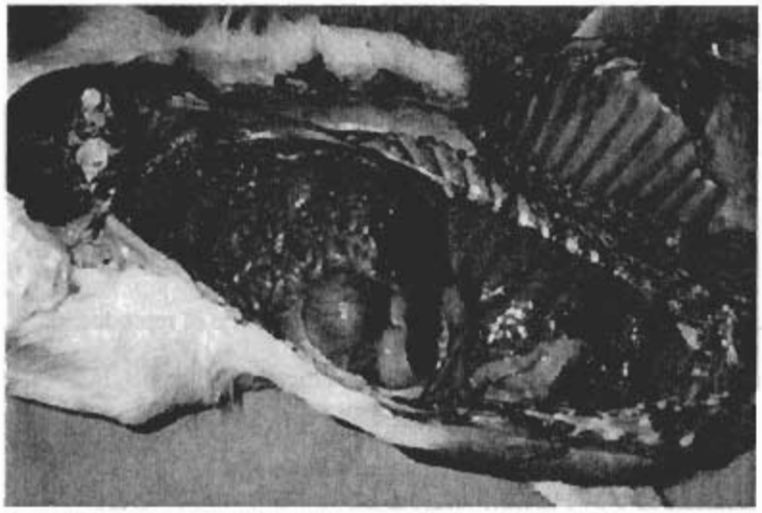

Fig. 4. In-situ internal organs of a properly prosected large ungulate during necropsy (photograph by T. J. Roffe).

   h. Reach under ribs; cut away diaphragm from right rib wall.
   i. At the head, cut the chin to separate the lower jaw.
   j. Cut all muscular attachments of the right lower jaw.
   k. Break the jaw outward and backward toward the right ear. The internal structures of the mouth and pharynx should be visible.
   l. Remove the tongue keeping it attached to the trachea and esophagus. This will require separating or cutting the hyoid bones at the back of the throat.
   m. Extend cut, separating tongue/trachea/esophagus to the chest.
   n. Mark muscles of right side ribs from the abdomen near the spine to the tongue/trachea/esophagus. Use rib cutters along this line to cut ribs.
   o. Mark muscles of right side ribs from the abdomen near the ventral midline to the tongue/trachea/esophagus. Use rib cutters along this line to cut ribs and remove right rib cage.
   p. Examine all organs in position.
   q. Remove thoracic organs by grasping trachea/esophagus near lungs, cutting along the spine, cutting along the sternum, and cutting structures along the diaphragm (aorta, esophagus, and caudal vena cava). Consider tying the esophagus before cutting if stomach is full.
   r. Remove liver by cutting gastrointestinal, kidney, and diaphragmatic attachments.
   s. Remove GI tract and spleen by cutting the distal descending colon, the diaphragmatic attachments, and the root of the mesentery.
   t. Separate the spleen from GI tract (located along stomach or rumen).
   u. For full exposure, remove the right (upside) half pelvis by cutting bone (ilium, ischium, and pubis) and soft tissue attachments. The entire urogenital tract and descending colon will be visible.
   v. Loosen urogenital tract and colon by first freeing kidneys, ureters, and ovary and uterine attachments. In males the reproductive tract is examined externally.
   w. Remove urogenital tract by grasping all organs and cutting soft tissue attachments in the pelvic canal.
2. Rotund or torpedo shaped mammals (bears, marine mammals, small furbearers).
   a. Place in dorsal recumbency (on their back).

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Box 3 (*continued*). General internal postmortem exam procedures.

b. Make midline incision as above from chin to pubis.
c. Reflect skin laterally from both sides of carcass.
d. Open the abdomen by making 2 flaps via a midline incision and creating one flap attached to each side.
e. Cut the mid lower jaw (chin) and remove tongue keeping attachments to esophagus and trachea.
f. Extend tongue/trachea/esophagus to chest as in 1.l and 1.m.
g. Mark muscles on both sides of the ribs from the abdomen, along the midsection of the ribs, to trachea/esophagus.
h. Remove ventral rib cage and sternum by cutting ribs along these lines.
i. Remove the remainder of organs as described in 1.q to 1.u.
j. Remove the ventral pelvis by cutting bone on both sides of the pubis and removing section of bone.
k. Remove urogenital tract as in 1.v through 1.w.

3. Birds
   a. Generally necropsy in dorsal recumbency and as above.
   b. Use blunt dissection to remove skin.
   c. Do not have diaphragm.
   d. Have air sacs.
   e. To remove rib cage must also cut coracoid and furcula, 2 bones attaching wings and breast muscle to main body. Use poultry shears.
   f. GI tract may include a gizzard, large paired ceca, and cloaca.
   g. Remove heart independent of lungs.
   h. Lungs are adhered to ribs and must be "peeled" from body wall.
   i. Some organ shapes differ from mammals.
   j. Reproductive organs vary greatly in appearance depending on stage of reproduction.
   k. Female reproductive system is unilateral, generally left side.
   l. Kidneys are long, 3 lobed, and tightly recessed in bony structures of the pelvis.
4. Organ Examination.
   a. Lungs.
      i. The esophagus is generally opened before examining the lungs.
      ii. Thorough palpation is more important than visualization when examining the lungs.
      iii. Open trachea and mainstem bronchi lengthwise starting at the larynx.
      iv. Continue opening distal bronchi until very small airways are reached.
      v. Cross section several areas of lung.
      vi. Content and mucosal surface both important to examine.
   b. Heart.
      i. Examine and open the pericardium first.
      ii. Open the heart in direction of flowing blood—Right atrium, right ventricle, left atrium, left ventricle.
      iii. Examine 3 surfaces (epicardium, endocardium, and myocardium) and 3 structures (valves, cords anchoring the valves, and the muscular ridges lining the ventricles).
      iv. The ratio of left to right ventricular free-wall is approximately 3:1 in most normal mammal hearts.
      v. Bird hearts are best opened by first cutting off the end of the heart to observe the relative size and shape of the 2 ventricles. The right ventricle will be a thin crescent around the cylindrical left ventricle.
   c. Liver and spleen.
      i. Examine surfaces.
      ii. Examine biliary tract and gall bladder, if present.
      iii. "Bread-slice" (series of parallel slices not quite through the organ) the liver and spleen and examine cut surfaces.
      iv. Bird spleens can be spherical or triangular. A single section through the organ is generally sufficient.
   d. GI tract.
      i. Open lengthwise along entire length or spot check at different points.
      ii. Esophagus and colon are part of the GI tract.
      iii. Examine all 4 stomachs of ruminants, single stomach of monogastrics.
      iv. Examine some section of duodenum, jejunum, ileum, and colon.
      v. In ruminants the colon is complex. Examine ascending colon, spiral colon, and descending colon.
      vi. GI parasites can be removed individually or by washing GI content through a sieve.
      vii. Gizzard malfunction can be estimated by the degree of excessive keratin on the surfaces.
   e. Urogenital tract.
      i. Adrenals are located at the front end of the kidneys. Cut in half to examine.
      ii. Slice kidneys lengthwise in half to visualize all structures.
      iii. Open bladder by making a small incision then inverting the bladder over gloved fingers.
      iv. Preserve ovaries and testes either whole or sectioned in fixative. Distal descending colon is usually examined with urogenital tract.

Table 2. Sample selection and preservation from field necropsy when entire carcass cannot be submitted and circumstances/necropsy findings suggest specific causes may be involved.

Sample	Projected tests	Method of preservation	Comments
	When microbial infections are expected		
Observed lesions	Microbiology	Frozen	Lesions (abnormal-appearing tissue): a portion of each should be saved frozen and fixed.
Heart	Bacteriology	Frozen	Entire heart from birds and small mammals; selected portions from larger animals.
Liver	Bacteriology	Frozen	Entire lobe from birds and small mammals; several pieces up to 2 cm^2 or larger in larger animals.
Blood/serum	Bacteriology/virology	Frozen	Serum also useful for serology.
Spleen	Bacteriology/virology	Frozen	Entire spleen from birds and small mammals; selected portions from larger mammals. Fix remainder.
Intestine (small fragment)	Bacteriology/virology	Frozen	Segments from middle or distal (ileum) of the small intestine.
Brain	Bacteriology/virology	Frozen	If animal exhibited abnormal behavior, save entire head; submit intact head to laboratory for removal of brain by laboratory personnel.
	When toxicants are suspected		
Lesions	As appropriate	Frozen	Lesions (abnormal-appearing tissue): a portion of each lesion should be saved frozen. Fixed tissue important.
Liver	Heavy metals (Pb, Tl)	Frozen	Entire liver from birds and small mammals; selected portions from larger mammals. Fixed tissue important.
Kidney	Heavy metals (Pb, Hg, Tl, Fe, Cd, Cr)	Frozen	Entire kidneys from birds and small mammals; selected portions from larger mammals. Fixed tissue important.
Stomach contents	Organophosphates, carbamates, plant poisons, strychnine, cyanide, mycotoxins	Frozen	Save entire contents. Samples to be checked for cyanide or H_2S must be placed in airtight container to prevent loss of these toxic gasses into the air.
Brain	Brain cholinesterase, organochloride residues, organomercuric compounds	Frozen	If brain is removed for chemical analysis, the brain must be wrapped in clean aluminum foil then placed inside a chemically clean glass bottle. Fixed tissue important.
Blood	Lead, cyanide, H_2S, nitrites	Frozen	Samples to be checked for cyanide or H_2S must be placed in airtight container to prevent loss of these toxic gasses into the air.
Lungs	H_2S, cyanide	Frozen	Samples to be checked for cyanide or H_2S must be placed in airtight container to prevent loss of these toxic gasses into the air.

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Table 2 (*continued*). Sample selection and preservation from field necropsy when entire carcass cannot be submitted and circumstances/necropsy findings suggest specific causes may be involved.

Sample	Projected tests	Method of preservation	Comments
	For microscopic study		
Lesions	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin	Lesions (abnormal-appearing tissue): a portion of each lesion should be saved frozen.
Liver	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin	Specimen portions should not exceed 6 mm in thickness.
Kidney	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin	Specimen portions should not exceed 6 mm in thickness.
Gonads	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin or Bouin's	Specimen portions should not exceed 6 mm in thickness.
Intestinal tract	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin or Bouin's	Snippet of stomach at the ileocecal junction, piece of duodenum (near the pancreas), and colon.
Brain, nervous tissues, eyes	Formalin-fixed material will be sectioned and stained	10% Formalin	Divide brain in half (sagittal); place one half in Formalin and save the other half frozen.
Impression smear	Can be made by touching glass slide to cut surface of any organ	Air-dry	Air-dried side can be used for many laboratory tests.
Heart, lung, skeletal muscle, lymph nodes, spleen, thymus	Specimen is fixed, sectioned, and stained for microscopic study	10% Formalin	Specimen portions should not exceed 6 mm in thickness.

SPECIMEN COLLECTION AND PRESERVATION

Timely response to wildlife disease outbreaks is important. Equally important is proper sample collection (Table 2, Box 4). The ability to identify the cause of mortality is highly dependent on sample quality. Great effort should be expended in locating the freshest carcasses possible. An example for collection of high priority species would be application of radio-transmitters with mortality sensors to facilitate location of fresh carcasses. Another method for obtaining the freshest possible specimens might include humane euthanasia of sick animals for postmortem exam. These specimens also provide the opportunity to collect fresh blood for analyses. Regular patrols to locate newly dead or sick animals should be a top priority during epidemic mortalities. If practical, fresh, unopened carcasses should be submitted to the laboratory chilled, generally using chemical ice packs. However, frozen specimens are better than no specimens at all. Several (>5) specimens over different time intervals need to be examined for many reasons. First, animals die of many causes. Some specimens may reflect diseases unrelated to what is causing epidemic mortality. Sufficient samples need to be obtained to identify the dominant mortality factors. Second, limitations of laboratory analysis may result in the offending agent not being found in every specimen that died. Many wildlife mortalities involve multiple factors that can only be detected over time. When possible, examining both sick and dead animals can provide a more complete picture of the disease event. Early signs of disease can be detected more readily in sick animals while dead animals may show late stage disease and opportunistic infections that, if examined solely, may complicate the picture.

In some cases, unaffected animals from unaffected areas may need to be examined for comparison. Attempts should be made to sample these animals in a nonlethal manner. However, should euthanasia be necessary, it should be conducted by qualified personnel in accordance with American Veterinary Medical Association approved guidelines (Beaver et al. 2001). Care should be taken to properly label all samples with a unique identification as well as location and collection date. If field necropsies are done, it is essential the labeling system ensure that laboratory results from samples can be referred to the specific specimen from which it was collected at necropsy. Strict hygiene should be used during sample collection to ensure personal safety and minimize exposure to potential zoonotic agents. At a minimum, protective clothing, footwear, and gloves should be used when handling carcasses or tissues. Biologists should thoroughly wash with water and soap after working with dead animals (Fig 5).

Box 4. Specimen collection.

1. Collect freshest specimens possible.
2. Use sharp knife or scalpel for tissue pieces, swab for surfaces and exudates, needle and syringe for fluids.
3. Collect all samples in separate containers except histopathology.
4. Prevent cross-contamination.
5. Use sterile sample plastic bags or tubes. Non-additive blood tubes (red top) can be used.
6. In general, freeze specimens unless they will get to the laboratory within 48 hours EXCEPT histopathology and parasitology samples.
7. Allow blood to clot. Centrifuge and remove serum. Freeze serum.
8. Label all samples from one specimen with the same unique number. Use indelible ink or pencil and labels that stay on in the freezer.

SPECIMEN SHIPMENT

There has been increased emphasis on security for shipment of air cargo. Thus, it is essential that biologists and receiving laboratories be familiar with U.S. Department of Transportation (DOT) and public health regulations involving shipment of biological samples by ground or air. Centers for Disease Control (CDC) and DOT regulations generally permit movement of "diagnostic specimens" although certain restrictions on packaging and select agents may be required. The Code of Federal Regulations is available by Internet (http://www.access.gpo.gov/nara/cfr/) and covers DOT shipping requirements for biological material (49 CFR 178) and "select agent" interstate movement and public health issues (42 CFR 72). Select agents are a unique list of pathogens provided by the CDC for agents considered a high risk for bioterrorism.

The first step in proper shipment of samples is to ensure they are properly packaged to avoid leakage and, in case of chilled or frozen samples, thawing or heating (Box 5). The best method is to work directly with the shipper to ensure samples are appropriately packaged and labeled. Some shippers will permit certain shipments whereas others forbid them. Although shippers will have the final word on acceptability of a particular shipment, knowledge of federal and state transportation regulations can assist in gaining their acceptance. Overnight shipment is the only practical method for perishable material. Time of shipping should ensure the package would arrive at the laboratory on a workday. Collection permits may need to be enclosed with the specimens. In other cases close coordination with appropriate law enforcement authorities may be necessary to avoid delays, particularly for international shipments. If the case is a legal matter, chain-of-custody forms will need to accompany the specimens. Under no circumstances should specimens be shipped without prior coordination with and consent of the receiving laboratory.

Fig. 5. Typical field equipment used when investigating wildlife mortality (photograph courtesy of T. J. Roffe).

SUMMARY

Wildlife population management requires knowledge of factors that affect population sustainability. Mortality is one of the most important of those factors. Without a clear understanding of the causes of mortality, decisions by managers of whether or how to intercede may be inappropriate. Wildlife biologists are usually the first to discover, assess, and respond to wildlife mortality. Biologists who make accurate, complete and timely field investigations, and proper collection and shipment of samples to a diagnostic facility are essential for an accurate diagnosis. In

Box 5. Packing samples for shipping.

combination with wildlife disease specialists, biologists can identify causes of wildlife mortality, detect long-term patterns in factors that affect the survival of populations, and take appropriate corrective action to minimize the impact of some mortality factors on wildlife populations.

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