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37

STRATEGIES FOR WILDLIFE DISEASE SURVEILLANCE

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Epidemiologic surveillance is defined by the Centers for Disease Control and Prevention (CDC) as the "ongoing systematic and continuous collection, analysis, and interpretation of health data". The objective of surveillance is to generate data for rapid response to the detection of a disease of concern to apply prevention, control, or eradication measures as well as to evaluate such interventions. This is distinct from disease monitoring, which usually does not involve a particular response to disease detection.

Surveillance for wildlife diseases has increased in importance due to the emergence and re-emergence of wildlife diseases that are threats to human, animal, and ecosystem health, or could potentially have a negative economic impact. It has been estimated that 75% of emerging human diseases are zoonotic in origin, of which the majority originate from wildlife (Taylor et al. 2001). However, there are unique challenges concerning wildlife disease surveillance such that disease and pathogens can be very difficult to detect and measure in wild animals. These challenges have been described previously (Wobeser 2006), but one of the primary issues is that disease in wildlife often goes unrecognized, especially in remote locations. Furthermore, sick and dead animals are very difficult to detect, as animals will disguise the signs of illness or hide when diseased. Carcasses from diseased animals are also rapidly removed by scavengers or will rapidly decompose, rendering them suboptimal for diagnostic purposes. There is also a lack of validated diagnostic tests for most wildlife disease agents as well as baseline data. The paucity of laboratory capacity with expertise in wildlife disease diagnostic investigation is also an impediment. Finally, surveillance networks for wildlife diseases that perform field investigations and report disease events are under-developed in most regions of the world.

Despite these challenges, a number of very important epidemiological surveillance projects have been ongoing or recently developed, and some examples are described in this chapter. The examples are mostly drawn from the experiences of the U.S. Geological Survey National Wildlife Health Center (NWHC) and are provided to illustrate the different surveillance strategies and sampling techniques that can be used and have proven successful. Some future directions for wildlife disease surveillance are also suggested.

SURVEILLANCE STRATEGIES

The first goal of any disease surveillance program is to define the objective(s), as the system established may vary depending on the desired outcome—that is, early detection or outbreak response; evaluation of disease management actions; determination of presence or absence of a disease or pathogen; for research or education; or a combination of these objectives.

While it is possible to achieve multiple objectives using the same system, very often the differing objectives may not be compatible. For example, early detection systems should be modified annually to respond to changing exposure risk factors, improved understanding of the epidemiology of the disease, and lessons learned from previous surveillance. However, from a research perspective this would preclude the ability for inter-annual comparability of results. Efforts should target different objectives to be as compatible as possible without compromising the primary goal. The establishment of accurate case definitions for wildlife diseases can also be a challenge, yet this is essential to ensure comparability among data collected by different groups.

Types of surveillance are commonly divided into two major categories, passive versus active and scanning versus targeted surveillance. Active surveillance involves actively searching for particular diseases or information; passive surveillance involves data collected from disease observations on an *ad hoc* basis. Scanning surveillance involves continuously searching for disease within a population, and targeted surveillance involves looking in selected high-risk subsets of the population. These techniques are often combined; for example, scanning passive surveillance involves the continual looking for and investigating wildlife mortality events.

Passive Surveillance

Passive surveillance takes advantage of previously collected data that are often obtained for different reasons but that are then used for surveillance purposes. Advantages of passive surveillance include cost-effectiveness and the ability to take advantage of convenience sampling and existing databases. Disadvantages include biased sampling and incomplete geographic coverage, precluding the ability to make statistical inferences about the population of interest. Maintenance and ongoing analysis of long-term datasets are necessary to determine baseline data for diseases and susceptible species before any perturbations to the established trends can be detected. Furthermore, wildlife population sizes are often unknown, and this lack of denominator information prevents calculation of disease prevalence and incidence and other basic descriptive epidemiologic parameters. An example of the use of passive surveillance was the ability to observe an unexpected increase in submissions of raptors to wildlife rehabilitators and diagnostic facilities that was determined to be due to West Nile virus infection (WNV) (Joyner et al. 2006; Saito et al. 2007).

A major use of passive surveillance is to evaluate factors relating to mortality events that can be useful in providing descriptive epidemiologic parameters and generating hypotheses regarding the impact of disease on wildlife populations. For example, a retrospective review of avian mortality events due to salmonellosis in the United States determined that this disease was a significant contributor to mortality in certain passerine species, and identified increased salmonellosis-related mortality in specific geographic regions (Hall and Saito 2008). A 20-year-old manatee (*Trichechus manatus*) database was used to analyze trends in watercraft-related mortality (Ackerman et al. 1995; Wright et al. 1995). Managers used this information to establish manatee protection zones and limit watercraft use in these zones to reduce manatee mortality. Long-term datasets at the NWHC were used to document the effects of lead ingestion by bald eagles (*Haliaeetus leucocephalus*) and waterfowl and provided the scientific information that resulted in the ban on the use of lead shot for waterfowl hunting in the United States (Franson et al. 1986; Friend et al. 1999).

Another use of passive surveillance is to combine two types of data—for example, water quality data and precipitation data with the incidence of red tides to determine whether environmental factors contribute to the emergence or persistence of these events (Landsberg et al. 2007). This analysis determined that red tides thrive in water with high salinity, which occurs in estuaries, especially during droughts. Manatees frequent estuaries because of the abundant grass beds; however, this feeding behavior exposes them to fatal concentrations of brevetoxin (Bossart et al. 1998), and this combination of information provided a better understanding of how red tide events affect manatees.

Morbidity and Mortality Investigations

Morbidity and mortality investigation of wildlife is a process whereby data are collected and analyzed to determine why an event occurred and if possible how to prevent or control this and similar events in

the future. It is the most commonly used type of passive surveillance. These investigations are dependent upon the discovery of sick or dead animals by the public and as a result are biased to events in highly populated or easily accessible areas, pathologic conditions that cause obvious clinical signs or death, or large, highly visible animals. To best determine the cause of wildlife mortality events, carcasses need to be examined by laboratories specializing in wildlife diagnostic investigations. Some species-specific surveillance programs have been developed; for instance, the Amphibian Research and Monitoring Initiative (http://armi.usgs.gov/; accessed March 27, 2011), which is designed to increase surveillance for amphibian mortality events.

As often as possible, disease investigations lead to a management response and are also included as part of larger, more comprehensive surveillance programs. For example, mortality investigations of species known to be susceptible to H5N1 highly pathogenic avian influenza (HPAI) represent an important component of the interagency surveillance strategy for early detection of H5N1 HPAI in migratory birds in the United States (Brand 2009). Enhanced mortality investigations may also be a component of the response to the detection of an important disease by other methods in a surveillance program, such that if HPAI was detected in a hunter-harvested bird, increased testing of dead birds for avian influenza in proximity to this detection would be instituted.

Disease investigations are characterized by the collection of information associated with the event, such as location, species and numbers of animals involved, time progression of the event, habitat type, recent weather, and potentially related human activity. This information is combined with necropsy findings and ancillary diagnostic evaluations (Fig. 37.1) and is used to determine the etiology, describe the circumstances surrounding an event, evaluate the ecological impact and risk to wildlife, human, or domestic animal health, and ultimately provide management recommendations. The investigation also represents a temporal and geospatial record of the particular event and will add to the baseline data, allowing the significance of a similar event in the future to be compared to past events. Furthermore, comparing it to findings from past events can more easily reveal a new disease. In this way, disease investigations provide the opportunity to discover novel pathogens. White nose syndrome in wild bats (Blehert et al. 2009), WNV in wild birds (Reed et al. 2003), avian vacuolar myelinopathy in American coots (*Fulica americana*) (Thomas et al. 1998), and *Perkinsus*-like organisms in frogs (Davis et al. 2007; NWHC unpublished data 2000) are a few recent examples of new diseases discovered in wildlife that resulted from mortality investigations.

In contrast, targeted surveillance does not require a full examination of the animals collected, thereby using fewer resources. Surveillance programs are often funded for the detection of a single disease agent, and so resources are focused on the work necessary to detect that disease. This was the case during the investigation of WNV in the United States. Thousands of dead wild birds were submitted to the NWHC for WNV testing but no further examination was possible, representing a missed opportunity. However, the selection of the type of diagnostic approach may allow for the identification of additional agents besides the targeted pathogen. If virus isolation rather than PCR is used, then additional agents can be identified through the targeted surveillance program. For example, other viruses such as Eastern equine encephalitis can be detected during WNV surveillance (Beckwith et al. 2002; Dusek et al. 2009).

The value of disease investigations contributing to our knowledge of long-term trends of wildlife diseases cannot be overemphasized. This value is realized when such data are used to predict and perhaps mitigate the affects of environmental factors such as global environmental change on wildlife health. Wildlife diseases such as avian botulism, WNV, avian cholera, and epizootic hemorrhagic disease (EHD) are affected either by seasonal availability of arthropod vectors and/or by host population density. Climate change could dramatically affect vector distribution or change migratory pathways or breeding seasons (Walther et al. 2002). In turn, these changes can affect the presence and distribution of diseases detected through clinical signs or mortality investigations. Using percentage of harvested white-tailed deer (*Odocoileus virginianus*) with hoof-wall growth interruptions as an indicator of the annual incidence of EHD, Sleeman et al. (2009) found that the incidence was greater in years with higher winter and summer average temperatures, and lower summer rainfall. They hypothesized that as temperatures continue to increase there will be more frequent and

Figure 37.1:
Pathologist at the U.S. Geological Survey's National Wildlife Health Center performs a necropsy on a gray wolf (*Canis lupus*).

severe outbreaks of EHD as well as spread to new geographic areas.

Success of large-scale disease investigation programs (regional, national, or global) depends upon the participation of many collaborators. Ideally, a surveillance network of trained field partners should exist to maximize the temporal and spatial coverage of the program. In the United States, the professionals most often involved are state and federal government employees who work for wildlife management or public health agencies, and occasionally personnel from universities and wildlife-focused nonprofit organizations. Although some mandates exist for reporting wildlife disease events, and attention to these events is received from highly trained personnel, it is often personal interest from individuals and groups that determines whether information or samples are submitted to a diagnostic laboratory. There is currently no legal requirement to report most wildlife diseases of management or conservation concern. However, professional training can enhance participation and improve quality of samples submitted by providing information on data and sample/carcass collection, shipping protocols, personal protection equipment (PPE), carcass disposal, and management recommendations. Professional workshops also provide the opportunity to explain why disease investigations are important and how the information collected is used to assist with management of wildlife populations and facilitate communication with stakeholders and the public.

In summary, mortality investigations serve as a "trigger event" to launch a more intense surveillance effort to contain or stop the progression or spillover of a disease. Information gathered is used to describe disease trends over space and time, and these long-term databases are used to generate hypotheses, predict future events, and illustrate the progression and persistence of diseases. As WNV progressed west from the East Coast of the United States, wild bird mortality data were used to indicate the presence of the virus in a new area as well as the change in wild bird species affected over time. By the time WNV arrived in the western half of the United States, the avian sentinel species changed from corvids to small passerines (Marra et al. 2004; NWHC unpublished data 1999–2004). Finally, for rarely encountered species such as cetaceans, much of what is known about

these species is gleaned from information collected during necropsies of the rare beach-cast animal.

Active Surveillance

Active surveillance is a proactive process of surveying for a particular disease, and is usually ongoing. Goals of an active surveillance program are typically (1) early detection of the introduction or occurrence of a disease in a given area or population so that timely and appropriate control measures can be taken; (2) demonstration of the absence of a disease; (3) assessment of the prevalence and spatial distribution of a disease to assist in determining disease management strategies; or (4) monitoring of a disease to determine epidemiological changes in response to disease management actions or other ecological or environmental changes (Thrusfield 1995). Active surveillance involves a more rigorous and complex approach to designing the program so that the results have statistical validity and unbiased inferences about the population of interest can be drawn. This often results in a relatively large sample size, which together with the increased logistics of capturing and handling free-living wildlife makes this form of surveillance expensive relative to passive surveillance. Because of this, large-scale active surveillance in free-living wildlife is usually limited to diseases of high consequence or global concern, such as chronic wasting disease (CWD), HPAI, bovine tuberculosis (*Mycobacterium bovis*), and Ebola virus outbreaks.

Simple probability-based surveillance methods include simple random sampling of the population of interest, stratified random sampling where defined subunits of the population are sampled based on knowledge of risk factors, systematic sampling, and cluster sampling (Ratti and Garton 1994). Random selection of individuals or units to sample within the statistical design framework is a key assumption for most probability-based methods of surveillance. However, randomness is problematic when conducting surveillance in free-living wildlife, as this assumption is often not met, and sampling is more opportunistic or "convenience sampling" (Anderson 2001). Environmental factors, species characteristics, methods of obtaining individuals for sampling, and human influences create a complex set of biases difficult or impossible to control in designing large-scale wildlife surveillance. Additional complexities in designing a probability-based surveillance program include lack of knowledge or definition of the population at risk, which is especially true of migratory wildlife. In many cases, the prevalence of the disease, or disease agent, is low, requiring relatively large sample sizes to detect an agent or determine significant changes in prevalence or distribution. The sensitivity and specificity of the tests used to determine infection or exposure is a factor that should also be considered in determining sample size requirements (see Aguirre Chapter 39, this volume). Statistical assistance and consultation should be sought in the design stage of an active surveillance program.

Targeted surveillance is a form of active surveillance in which statistical inferences to the population of interest are limited. In targeted surveillance a cohort of the population of interest is targeted for sampling because it has a higher risk for exposure or is more susceptible, or identification of infection or exposure in an individual is easier or more reliable than in the rest of the population. In many regards, targeted surveillance and sentinel surveillance using free-living wildlife are similar in concept, and the terminology is often used interchangeably. For example, several waterfowl species—*Cygnus* spp. (Newman et al. 2009), Eurasian pochard (*Aythya ferina*) and tufted duck (*A. fuligula*) (Keawcharoen et al. 2008)—have been referred to as sentinels for the occurrence of H5N1 HPAI because of their high susceptibility (i.e., mortality) to this virus as well as visibility on the landscape. Surveillance for CWD often targets animals displaying typical clinical signs, such as neurological deficits and emaciation (Samuel et al. 2003). In these examples, the primary goal of surveillance is detection of the disease in an area, rather than a determination of prevalence or distribution. Selection of the targeted populations is to optimize the likelihood of detecting the disease. Inferences about the population of interest from finding one or more positive animals are limited largely to the knowledge that the disease or agent is present, and further studies are needed to elaborate on the prevalence in the population. Under some conditions, selection of target sub-populations can be based on the efficiency of obtaining samples, which also may increase cost-effectiveness.

A nationwide surveillance program for the early detection of the introduction of H5N1 HPAI to the United States by wild birds was initiated in 2006 due to the increased recognition of the potential role of

migratory birds in the long-distance expansion of this virus (USDA and USDI 2006). Multiple sampling methods were employed. The first stage applied unequal probability random sampling that was weighted by geographic region. Emphasis was placed on collecting samples from migratory birds in Alaska and the lower Pacific Flyway states because of the number of waterfowl and shorebird species that are known to migrate between North America and Asia, including migratory birds from regions in Asia where H5N1 HPAI was occurring (Brand 2009). Molecular studies of 38 low-pathogenicity avian influenza viruses isolated from Alaska during 2006 and 2007 as part of this surveillance program showed that nearly half of the viruses had at least one gene segment more closely related to Asian than North American strains of viruses (Koehler et al. 2008), indicating a higher degree of intercontinental viral genetic exchange in Alaska than previously reported (Krauss et al. 2007). A total of 72,320 wild birds were tested during three surveillance years between 2006 and 2009 using live-captured and hunter-killed birds; this represents one of the largest wildlife disease surveillance projects undertaken (NWHC, unpublished data 2006–2009). It is important to regularly evaluate large-scale active surveillance programs to ensure that goals are being met as well as to determine cost-effectiveness. For example, results from HPAI surveillance have increased our understanding of the epidemiology of avian influenza viruses that will be useful in the design of new and more effective surveillance programs (Munster et al. 2007).

WILDLIFE SENTINELS FOR HEALTH AND DISEASE

The concept of using sentinel animals as a surveillance tool has been widely applied for both infectious diseases and environmental toxins (Thrusfield 1995), though is probably underused (Rabinowitz et al. 2005). In its broader sense, a sentinel can be defined as a susceptible animal (or a sentinel unit as a susceptible population) used to detect or quantify the presence or occurrence of a pathogen, disease, or other environmental hazard. The utility of a sentinel is its ability to serve as an indicator of the presence or absence of an agent in a given area in a more timely, sensitive, visible, or cost-effective manner than other types of surveillance. This is because sentinel animals are either more at risk, sensitive, or susceptible to the specific agent than the species or population of concern; effects of the agent are more easily observed or occur earlier in sentinels than in target populations; sentinels are more easily observed and sampled than other animals; sentinels are the actual source of the agent for the target population; or it is logistically more cost-effective than other forms of surveillance.

Halliday et al. (2007) lay out a framework for evaluating the utility of sentinel animals for infectious diseases based on characteristics of the pathogen, the target population, and the sentinel species or population. Depending on the specific objectives of the surveillance and its ecological context, critical attributes of the sentinel system that must be considered include (1) sentinel response to the pathogen or agent, (2) relationship between sentinel and target populations, and (3) routes of transmission.

Wildlife sentinels in particular have been used to determine the presence of disease agents for zoonotic diseases in which the human population is the "target" of concern (e.g., WNV in crows [Eidson et al. 2001] and sylvatic plague [*Yersinia pestis*] in carnivores [Willeberg et al. 1979]) as well as for diseases of domestic animals and livestock (e.g., *rinderpest* in African buffalo [*Syncerus caffer*] [Rossiter 1994]). However, sentinels have also been used for diseases of concern to wildlife conservation (e.g., the presence of canine distemper virus in domestic dogs in close proximity to wild African carnivores [Roelke-Parker et al. 1996]).

Other examples include use of wing-feather clipped mallards (*Anas platyrhynchos*) as sentinels to determine the onset and course of avian botulism (*Clostridium botulinum* type C) on wetland units (Rocke and Brand 1994). The objective of this work was to determine the site-specific environmental factors related to botulism toxin production and transfer to birds. Using free-flying birds as sentinels for botulism posed uncertainties as to whether ingestion of toxin occurred at the site of morbidity or mortality, or on adjacent wetlands—hence the use of wing-clipped birds. Close monitoring and rapid removal and replacement of moribund and dead sentinels also enabled a quantitative assessment of the magnitude of mortality and relative availability of toxin. Similarly, coyotes (*Canis latrans*) and other carnivores have served as effective sentinels for sylvatic

plague in wild rodent populations (Willeberg et al. 1979) and have been used to alert public health agencies to the risk for plague infection in humans. Frölich et al. (1998) demonstrated the utility of red foxes (*Vulpes vulpes*) as sentinels for rabbit hemorrhagic disease virus through their antibody response to the accumulative effect of consumption of infected rabbits.

In certain situations, animal sentinels are deliberately placed in the field to detect infection or exposure to agents. Confinement in cages or restriction of movements allows access to these sentinels for sequential observations and sampling, as well as the ability to account for the sentinel population at risk, quantify morbidity and survival rates, and examine time-series responses. Rocke et al. (2002) used a combination of wild-caught American coots and captive-reared, wing-clipped mallards that were penned as sentinels on a North Carolina reservoir to detect the onset and course, potential source, and etiology of an unknown disease agent causing avian vacuolar myelinopathy (Thomas et al. 1998). In Hawaii, Atkinson et al. (1993) used sentinel chickens and canaries exposed in cages hung in the forest canopy to monitor the transmission of avian pox and avian malaria to determine specific locations and elevations where disease transmission in endemic forest birds was occurring (Fig. 37.2). The deliberate exposure of wild or captive-raised sentinels as described above offers several advantages over the use of "natural" or free-living wildlife sentinels, but also requires precautionary measures. Care should be taken that other diseases potentially affecting wildlife are not introduced into wild populations by the sentinels, and that they do not serve as reservoir or amplification hosts for diseases present in wild populations.

Wildlife sentinels have also been used as indicators of ecosystem or environmental health (NRC 1991). For example, mink (*Neovison vison*) are often used as sentinels for persistent and ubiquitous contaminants such as mercury and polychlorinated biphenyls as they are widely distributed, abundant, and regularly trapped, making them an excellent model to monitor environmental pollution on temporal and spatial scales (Basu et al. 2007). Furthermore, as high-trophic-level, piscivorous mammals, mink bioaccumulate appreciable concentrations of pollutants, increasing the detection of these compounds. For the same reasons, several marine vertebrate species make excellent sentinels for marine ecosystem health (Aguirre and Tabor 2004; Tabor and Aguirre 2004).

DATA MANAGEMENT, RESPONSE, AND COMMUNICATIONS

Determination of data to collect and systems to use to capture field data is necessary before beginning surveillance. At a minimum, data on sample identification, species, date, age, sex, and location should be collected. Data fields must be standardized to allow comparability, although such standards are rarely used in wildlife disease surveillance. The traditional paper data card is being replaced by PDAs or smart phones, often with GPS capabilities that allow for the electronic capture and transfer of data to a database. This results in fewer transcription errors, among other advantages. Finally, a database system to track, store, retrieve, analyze, and disseminate information is an essential component, and there are a number of database formats, such as SQL server, that allow Internet-based systems with Web access. Response plans should be in place for all diseases for which active surveillance is being conducted. These plans define the actions that will be taken should the disease be detected. It should include communications plans; assessment and monitoring surveillance plans; specific regulatory, disease prevention, control, or eradication actions that may be taken; and how success will be measured.

TYPES OF SPECIMENS

The type of diagnostic samples collected will be determined by the surveillance technique and sample transport requirements as well as the goal of the surveillance effort. Samples can range from whole carcasses, specific biological samples such as blood, the measurement of biomarkers, use of proxy species, or simple observation of clinical signs, to name a few. Fresh carcasses are advantageous as they provide the maximum amount and diversity of biological materials for diagnostic investigation, which is particularly useful if the etiology is unknown. As discussed previously, moribund and dead wild animals can be very difficult to find, and active searching for carcasses, or "carcass sweeps," in geographic areas at risk for exposure can be useful. These searches are subject to sampling bias and are dependent on species, terrain, and disease of interest (Wobeser 2006). However, the use of volunteer observers, or "citizen scientists," to

Figure 37.2:
Federal biologists set up cages containing sentinel chickens and canaries in the forest canopy in Hawaii to monitor the natural transmission of avian pox and avian malaria to determine specific locations and elevations where disease transmission in endemic forest birds is occurring.

collect data on house finches (*Carpodacus mexicanus*) with clinical signs of mycoplasmal conjunctivitis illustrates the usefulness of engaging the public in tracking the spatiotemporal spread of a disease on a large geographic scale (Dhondt et al. 2005).

There has also been increasing interest in the use of syndromic surveillance as part of early detection systems. Syndromic surveillance applies to surveillance using health-related data that precede diagnosis and signal a sufficient probability of an outbreak to warrant further investigation (Buehler et al. 2003). The feasibility of detecting bioterrorism events by investigating wildlife mortality is being explored, especially as several bioterrorism agents of highest concern are also wildlife diseases. Consequently, an unusual die-off of a wildlife species known to be susceptible to a particular bioterrorism agent may be an early warning of risk to human health, especially if clinical signs manifest in animals before humans (Rabinowitz et al. 2006). However, syndromic surveillance systems in wildlife have not been rigorously evaluated.

Collection of animals by lethal methods for diagnostic sampling can also be performed, and with appropriate design this method may eliminate some of the sources of bias and allow for more random sampling. This method is usually employed when random sampling is required to determine the prevalence or geographic distribution of the disease of interest. It also allows for the collection of the widest variety and optimal tissue samples for diagnostic purposes. However, this method can be controversial and cannot be used for threatened and endangered species.

Sources of wildlife convenience samples for carcasses, live animals, and other biological materials include hunter-harvested animals, road-killed animals, animals brought to wildlife rehabilitators, and ongoing research projects. The non-randomness of convenience samples militates against straightforward inference from sample to population, but they have been used for recent surveillance projects such as H5N1 HPAI in migratory birds (Brand 2009).

Radiotelemetry tracking of animals, particularly if fitted with mortality sensors that facilitate the recovery of dead animals, provides unique opportunities to determine the cause of mortality due to the availability of fresh carcasses as well as the population-level effects of disease as the population size at risk is known. For example, an outbreak of EHD in a radio-collared population of white-tailed deer allowed the

detection of an event that would have gone undetected as well as the determination of an accurate mortality rate (Beringer et al. 2000). In addition, satellite telemetry, though expensive, can provide local and long-range movement data for migratory animals. This has been used to provide valuable movement data for species that are natural reservoirs for important pathogens such as birds with HPAI and bats with Nipah virus (Epstein et al. 2009). Not only are these data important for understanding host range, but they also provide expanded spatial information about disease distribution that allows for broader risk assessments.

Biological samples for surveillance purposes can also be collected from live animals: either samples can be collected opportunistically during routine operations, or animals can be specifically captured or handled for sampling purposes. The types of specimens that can be collected from live animals include blood for serological or molecular analyses, feces for parasitological evaluation, feathers or pelage for heavy metal analysis, as well as soft tissue or bone biopsies, among other samples. Fresh urine, feces, and feathers may also be collected without capturing an animal. The diagnostic information available from live-captured samples can be more limited compared to postmortem examination of whole carcasses as well as more technically challenging and expensive to obtain. However, this can be a useful technique that allows targeting of specific populations or when lethal collection is not feasible or desired (Aguirre et al. 2002).

Exposure to noxious substances can be detected by measuring physiological indicators or biomarkers. Examples include measurement of enzymes such as cholinesterase and delta aminolevulinic acid dehydratase to indicate organophosphate or carbamate pesticide exposure, and lead poisoning, respectively (Friend and Franson 1999). In addition, activation of the hepatic enzyme cytochrome P450 occurs after exposure to various compounds such as polyaromatic hydrocarbons. Many of these physiological responses lack specificity and will occur after exposure to a variety of compounds, limiting their usefulness in determining etiology. However, they can be useful in monitoring the long-term health of wildlife populations and ecosystems exposed to contaminants. Surveying for cytochrome P450 levels in sea otters (*Enhydra lutris*) after the Exxon Valdez oil spill has been used to determine population health and evaluate progress toward near-shore ecosystem recovery (Peterson et al. 2003).

The use of proxy indicators or species takes advantage of the trophic relationship in which predators or scavengers are examined for evidence of the disease agent in the prey. This method uses the fact that predators will be exposed to a large sample of prey animals as well as that predators are generally longer-lived than prey. A recent study investigating the potential of coyotes as sentinels for *M. bovis*, which is present in white-tailed deer in northeastern Michigan, found that by focusing on coyotes rather than deer, 97% fewer animals were sampled and the likelihood of detecting *M. bovis* increased by 40% (VerCauteren et al. 2008).

Disease surveillance of hosts that are not the species of most concern or the direct target of management actions can be a useful technique in assessing risk to the target wildlife population or in assessing the impact of management interventions. For example, surveillance for canine distemper virus in domestic dogs has been performed to assess risks to wild carnivores in contact with their domestic counterparts as well as to evaluate vaccination campaigns (Bronson et al. 2008; Cleaveland et al. 2000, 2006).

The questionnaire is a common tool used in public health and agriculture to obtain surveillance data (Thrusfield 1995). However, this technique has not commonly been used for wildlife disease surveillance, as free-ranging wildlife populations are usually not closely associated with humans. Surveys of demographic groups who have regular contact with wildlife, such as hunters or wildlife rehabilitators (Kalish et al. 2005; Schopler et al. 2005), can be a useful technique. Furthermore, this technique can be useful in evaluating health risks to wildlife populations from humans or domestic animals. Guerrera et al. (2003) conducted interview questionnaires of villagers living in close proximity to mountain gorillas (*Gorilla beringei beringei*) in Bwindi Impenetrable Forest National Park, Uganda, to estimate the prevalence of infectious diseases in this human population and consequently to evaluate the risk for transmission from humans to gorillas. Questionnaires are also useful for meta-analyses (i.e., the collection and analysis of data from a variety of sources for the purpose of integrating the findings; Gordis 2000) and can be especially useful for obtaining unpublished data. The World Organization for Animal Health (OIE)

regularly sends questionnaires to compile wildlife health data from participating countries into a central database.

Surveillance approaches for diseases can also involve detection of disease-causing agents in the environment—the air, water, soil, or other environmental matrices that can serve as sources of exposure to infectious agents or contaminants. Enteric diseases in particular are excreted by infected animals into the water or soil, and can persist for variable but sometimes extended time periods, depending on the pathogen and the physical, chemical, and biological properties of the environmental matrix. Fecal material itself can be used as an environmental surveillance tool, and was used in the H5N1 HPAI early detection surveillance in the United States (USDA and USDI 2006). Advantages of using environmental samples include the relative ease of obtaining samples, the ability to collect relatively large sample sizes, and the site-specific information on the distribution of the disease and exposure risk. However, numerous factors will affect the reliability of this method for detecting pathogens. These include knowledge of factors such as the modes of transmission and excretion of the agent; survivability or persistence of the agent under various environmental conditions; diagnostic methods, quantification methods, detection limits specific for the agent and validated for the environmental conditions under which samples were collected; and the appropriate sampling design. Other disadvantages include lack of assurance of host species when multispecies flock or herd is tested as well as the limited data that can be collected on specific animals, such as age and sex. However, for closely monitored populations in which individuals can be identified and tracked, these detailed demographic data may be available. Sleeman et al. (2000) were able to conduct detailed parasitological surveys of mountain gorillas in which the prevalence of different parasites could be compared among groups, and between age and sex as these animals were closely observed, allowing environmental fecal samples to be linked to specific individuals.

FUTURE DIRECTIONS

Disease prevention is the desired method to protect the health of wildlife populations, as once a disease has been introduced into a population it can be very difficult, if not impossible, to control or eradicate (Wobeser 2006). There are few effective wildlife disease management tools available (e.g., population reduction, use of vaccines or other biologics, and environmental modification), but they are expensive, often lack any assurance of success, and can be unpalatable to the general public. To increase the probability of successful wildlife disease management, future surveillance efforts should be based on risk analysis, investigation of potential exposure pathways, and improved knowledge of reservoirs of potential emerging pathogens (Haydon et al. 2002). New molecular techniques have opened up avenues for pathogen discovery not previously available (Lipkin 2008), and application of spatially referenced databases such as GIS allows for risk assessments that can assist in targeting surveillance to high-risk populations and geographic locations (Sleeman 2005). Integration and analysis of real-time data from a variety of sources, including human and animal health data with climatic, ecological, hydrological, geological, and socioeconomic data, among other sources, to determine drivers of disease emergence and generate predictive models will help direct resources to geographic areas and populations, so-called hotspots, with the greatest need (Jones et al. 2008). Increased global capacity to detect, diagnose, and provide robust and rapid responses to wildlife disease outbreaks and emerging diseases will also be critical in this effort.

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