TRAINING MANUAL ON WILDLIFE HEALTH INFORMATION MANAGEMENT

Sixth Cycle

Workshop for WOAH National Focal Points for Wildlife

World Organisation for Animal Health
Founded as OIE

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Web addresses and links to previous workbooks referred to throughout this manual:

Training Manual on Surveillance and International Reporting of Diseases in Wild Animals: 2nd Cycle Workshop for WOAH National Focal Points for Wildlife

https://www.woah.org/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/WGWildlife/A_Training_Manual_Wildlife_2.pdf

Training Manual on Wildlife Health Risk Assessment in Support of Decisions and Policies

https://www.woah.org/fileadmin/Home/eng/Internationa_Standard_Setting/docs/pdf/WGWildlife/A_Training_Manual_Wildlife_3.pdf

Training Manual on Wildlife Diseases Outbreak Investigations: 4th Cycle Workshop for OIE National Focal Points for Wildlife

https://www.woah.org/fileadmin/Home/esp/Internationa_Standard_Setting/docs/pdf/WGWildlife/A_Training_Manual_Wildlife_4.pdf

Training Manual on Wildlife Health Information Management: 5th Cycle Workshop for WOAH National Focal Points for Wildlife

https://www.woah.org/fileadmin/Home/esp/Internationa_Standard_Setting/docs/pdf/WGWildlife/A_Training_Manual_Wildlife_5.pdf

FOREWORD

WOAH Collaborating Centre for Research, Diagnosis and Surveillance of Wildlife Pathogens

This workbook has been developed for the 5th Cycle Training for WOAH Focal Points for Wildlife. During the trainings associated with the previous cycles, focal points were surveyed to determine the topics they were most interested in receiving future training. From that survey, it was evident that there was a need to provide training in wildlife health information management. Despites its practical importance, this topic is not well represented in the wildlife health literature, and our goal was to help to fill this gap with the material presented within this workbook.

The workbook is structured to follow a logical progression associated with wildlife health information management. We begin by detailing why it is important to share wildlife health information. We examine common challenges associated with information sharing and explore potential opportunities to overcome these challenges. Next, we examine general principles to establish a wildlife health information network and provide some case studies of real-world examples of successful networks. This is intended to address who should be included in a network, and how to establish the network structure for acquiring wildlife health information. We then progress to discussing what type of data should be collected and describe some best practices for data management and curation. This section explores what to collect and share with regards to wildlife health information. Finally, we conclude with a discussion on data dissemination with a particular focus on using Geographic Information Systems (GIS) as a tool for disseminating wildlife health information. This last section describes how to share wildlife information, which is generally the ultimate goal of wildlife health information management.

We intend this workbook to provide the basis for developing a general understanding of wildlife health information management; however, this topic is broad in scope, and application of the presented concepts is often highly dependent on local conditions, challenges and governmental structures. Therefore, we present the material at a general level drawing upon the literature, expert opinion and personal experience. Throughout the material, we provide references and electronic links to additional resources for readers who desire to develop a more in-depth understanding of the topics presented.

Wildlife health information management is becoming increasingly important component of surveillance and management of wildlife diseases. We hope this workbook will help focal points in maintaining and/or improving the health of wildlife in their countries for the benefit of wildlife, domestic animal and human health.

WILD ANIMAL DISEASE SURVEILLANCE

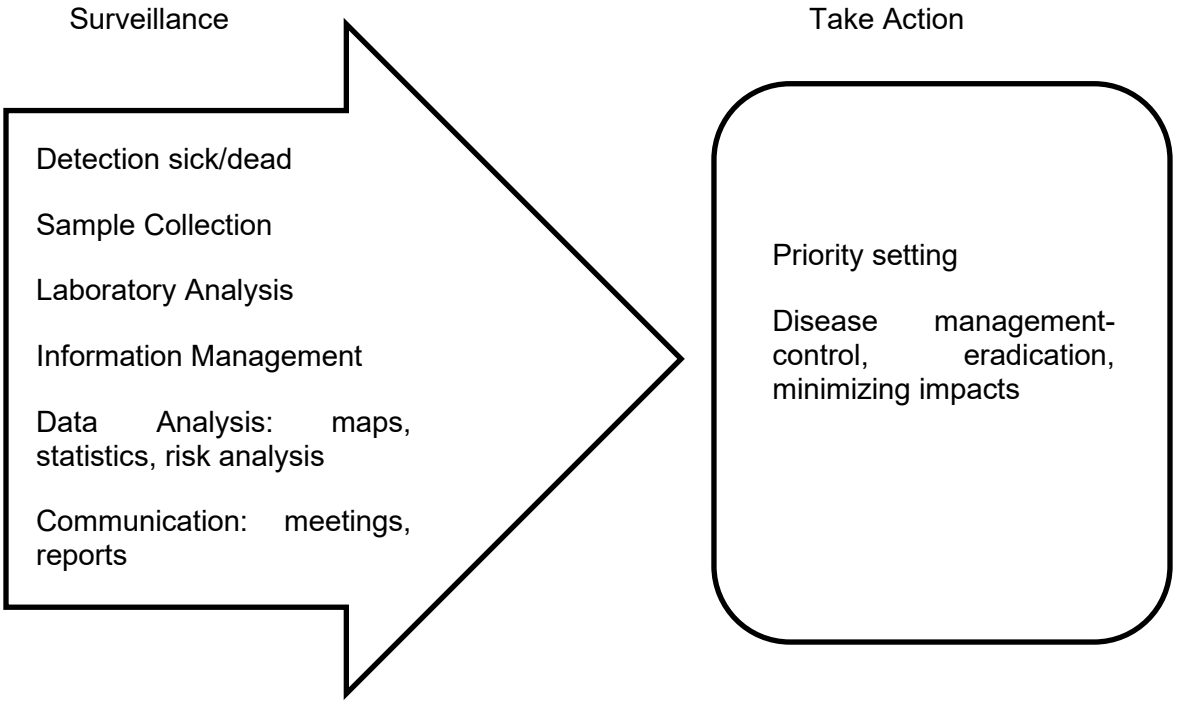
Surveillance for wild animal pathogens is the foundation of a comprehensive national wildlife health program. Surveillance is defined as “the systematic on-going collection, collation, and analysis of information related to animal health and the timely dissemination of information to those who need to know so that action can be taken” (WOAH *Terrestrial Animal Health Code*). It is the basis of a nation’s wildlife health program because it provides the essential information to select and implement the appropriate actions to promote wildlife health including prevention, detection, risk analysis, or management. Key attributes of successful surveillance include:

1. Surveillance is an on-going activity - investigation and vigilance for pathogens in wildlife and the diseases they may cause is most effective if it is continuous

2. Surveillance involves both the collection and analysis of data and information

3. Surveillance includes communication of the results of data collection and analysis to the full range of people, agencies and institutions that need the information

4. Surveillance creates information for action.



Through wildlife pathogen surveillance a country can detect the presence of pathogens in its wild animal populations, assess the geographic distribution of infection, and determine potential risk to important resources. Without surveillance new, emerging diseases may go undetected and the extent to which a population has been affected by some disease/pathogen will remain unknown (the prevalence of disease within the population). Surveillance provides the information that WOAH Wildlife Focal Points need to carry out his or her international responsibilities for disease

reporting which is critical for helping countries evaluate pathogen associated risks to their human, wildlife, agricultural populations.

Infrastructure needed for a successful surveillance program include not only a wild animal observation network and veterinary diagnostic laboratories but also information management, analysis, and communication systems. Each of these components are important when a country decides to respond to a disease outbreak and take management actions. Thus, wildlife disease surveillance not only promotes wildlife health, but also contributes to the national capacity to manage urgent domestic animal health events, particularly transboundary events. The importance of surveillance of wildlife cannot be over-emphasized.

FORMS OF PATHOGENS DISEASE SURVEILLANCE

There are two primary types of pathogen surveillance- general surveillance and targeted surveillance. Both forms of pathogen surveillance are important in a national wildlife health program. General surveillance scans wildlife populations to ascertain what pathogens exist. Targeted surveillance focuses on determining the presence or absence of a particular pathogen in wild populations. General or "scanning" wildlife pathogen surveillance is a particularly critical component of a national wildlife health program as it helps maintain national vigilance for emerging diseases associated with wild animal pathogens

Many aspects of animal health surveillance are described in Chapter 1.4 of the WOAH's *Terrestrial Animal Health Code*. However, some aspects of pathogen and disease surveillance in wild animals are unique and require additional consideration. Wild animals have no owners or attending veterinarians to recognize illness. The routine diagnostic tests for pathogens and diseases developed for domestic animals may or may not be valid for wild animal species. Wildlife biologists and ecologists are needed to provide ecological data on populations and other aspects of wildlife biology and to help analyze, interpret and communicate the results for a wildlife disease surveillance program. Additionally, in wild animal populations, traditional probability-based methods for selecting samples (Terrestrial Animal Health Code, Chapter 1.4.4) seldom can be used because of practical problems of access to wild animals, difficulty in defining a population, and lack of accurate information about population sizes and structures. Therefore, specific techniques for wild animals must be used. For example, despite probability-based sampling being impractical for selecting individual wild animals for testing, it may be possible to conduct probability-based sampling of geographic areas containing wild animals when samples are being collected across a spatial extent (see 4th Cycle Training Manual on Wildlife Disease Outbreak Investigations). But in general, most samples in wildlife pathogen surveillance will be non-random and will be based on what is possible to achieve given the difficulties of securing samples from wild populations (often called "convenience sampling"). This will affect the analytical approaches that can be applied to the surveillance data, the nature of the conclusions that can be drawn from the data, and therefore should be limited to the extent possible. Nonetheless, such surveillance remains a powerful and essential tool in national and international management of animal and human health and should be carried out in every country.

General Surveillance Program for Wildlife Pathogens

As noted earlier, wildlife pathogen surveillance consists of multiple components that must be coordinated in order to provide a cohesive surveillance program. Each of these five components involves different people with different training and expertise and, often, from different branches of government or from non-government organizations or universities. Each of these components is described in more detail in the following sections.

Detection of pathogens and diseases

Most general wildlife pathogen surveillance programs are based on examination of wild animals found ailing or dead. Thus, a critical component of a general surveillance program for wildlife

pathogens is a network of people who are likely to encounter dead or sick, wild animals (see additional recommendations for building a network in 5th Cycle Training Manual on Wildlife Information Management). These same people or others must be willing to report these findings to appropriate authorities and/or trained to safely collect these dead wild animals and transport them to an animal disease diagnostic laboratory. Alternatively, these individuals may be trained to necropsy such animals in the field and send the correct samples to the laboratory. The question is who can carry out this work? The answer to this question will undoubtedly differ among countries, but a successful program will require a network of people who spend time in areas inhabited by wild animals, can recognize disease or mortality events, and who are knowledgeable about reporting dead or sick wildlife to persons responsible for ensuring adequate specimens are sent to an appropriate laboratory. Thus, the programs responsible for wildlife pathogen surveillance must recruit the interest and continued cooperation of a wide range of people who spend time in wild animal habitats. Such people can include government wildlife officers and biologists, usually associated with ministries, departments or agencies (federal, state/province, regional) responsible for wildlife management. These people require permission and encouragement from their employers to participate in the surveillance program. Other potential participants include hunters, fishermen, naturalists, university scientists, non-government conservation organizations, wildlife rehabilitators and the general public. Those responsible for the wildlife pathogen surveillance program will need to allocate time and resources every year to maintain and support this network of people engaged in detection of sick or dead wild animals and the transport of specimens to laboratories.

To obtain active participation in a surveillance program, participants often need encouragement to prioritize surveillance activities. This may be as simple as showing the value of their contributions through regular updates on the program and its findings. They may also require financial assistance to perform surveillance activities. The surveillance program coordinators should be prepared to provide technical assistance in the form of a knowledgeable point of contact or through periodic training sessions.

Identification of pathogens and diseases

Once dead or diseased wild animals are detected, they must be examined to determine why they are sick or dead, and what pathogens they may carry. Ideally, this work is carried out by animal pathologists in appropriately equipped animal disease diagnostic laboratories with access to microbiologists, molecular biologists, virologists, parasitologists and toxicologists. During diagnostic testing, laboratories must consider a wide range of viral, bacterial, protozoal, fungal, and metazoan infectious pathogens, as well as various toxins and environmental contaminants and poisons. Laboratories with these capabilities are often associated with a country's ministry/department/agency responsible for agriculture, domestic animal health and Veterinary Services. Thus, the ministries or agencies responsible for wildlife and the ministries or agencies responsible for veterinary diagnostic laboratories usually must collaborate closely on any wildlife pathogen surveillance program if these functions are separated.

Information management

Any disease surveillance program must have a system to record the information it generates about disease or pathogen occurrences so that the information can be used to achieve the objectives of the surveillance program and appropriate actions can be taken. The most suitable approach to management of surveillance information is some form of computerized database or

archive of surveillance data (see 5th Cycle Training Manual on Wildlife Health Information Management). While it is possible, initially, to manage such information with spreadsheets or database software available for personal computers, such software quickly becomes inadequate with large amounts of data. Thus, it is useful to recruit people with knowledge and skills in computer database design and management prior to initiating a surveillance program. This will ensure the proper infrastructure is in place to efficiently and accurately capture the surveillance data to effectively support the other components of the national surveillance program.

Analysis and communication

In order to gain an understanding of the current disease situation for wildlife within a country, the "signal must be separated from the noise" inherent in the information collected from general surveillance. Statistical and epidemiological experts are generally familiar with this type of data and the analyses needed to examine and summarize it. The required analyses may involve estimating intensity metrics such as prevalence, presence/absence of a disease on a landscape, likelihood of a population being disease free, or other epidemiological parameters of interest. However, in contrast to domestic animal surveillance data, wildlife surveillance data will also likely require wildlife biologists and ecologists who can put the data into the proper context (e.g., provide information on species occurrence, management practices, intra- and inter-specific interactions, habitat needs, etc.).

Communication of surveillance results is critical and cannot be overstated in its importance. Not only is it important for disseminating necessary information to inform disease management actions, but also helps to sustain interest in participating in the surveillance network. The participants in the wildlife disease surveillance program, including government and non-government groups, typically expect to see the results of the surveillance program through various communications from the program coordinators.

Having a clear communication plan established for the surveillance program prior to the onset of data collection, is recommended to provide timely dissemination of information and to provide the requisite guidance for public, inter and intra-agency communication should a significant disease outbreak be detected via surveillance efforts. Due to variances in governmental structures, the responsibility of managing pathogens and disease in wild animals is often not clearly assigned. Certain pathogens may fall under the Ministry of Health, while others may fall under the Ministry of Agriculture and Veterinary Services. Responsibility for managing wild animal populations may frequently fall under the Ministries of Environment, Forestry or Fisheries. There may be confusion as the which branch or branches of government should be responsible for outbreak investigation, response and communications. Therefore, successful surveillance programs are based on having pre-established inter-ministerial or inter-departmental collaborations which agree on objectives and define the roles and responsibility of each entity, including communication to the public. A good communication plan should also ensure that agricultural or public health officials are contacted in the case an agricultural or zoonotic disease is discovered, or if there is any risk to food safety. Environmental officials should be informed in the event of a toxic chemical or other environmental contaminant is detected. Communication efforts should also ensure wildlife focal points are providing wildlife disease surveillance data to WOAH through the WAHIS-WILD interface to promote situational awareness and international coordination of wildlife disease management. It is also essential that communication and information that is made available to the public or non-scientific members of the surveillance network should be prompt, accurate, and in non-scientific jargon. Lastly, if possible, employing or seeking guidance from communication specialists can greatly enhance the effectiveness of communication efforts.

Taking action

Surveillance should provide data or information for action. Inherent in conducting surveillance is the need to provide the requisite knowledge to protect the health of wildlife and potentially domestic animal and human populations. The choice of the level of response to the detection of pathogens or disease in wild populations will be highly varied depending on the pathogen and host species involved, the threat to a country's resources, and the availability of resources. We describe below various activities that may be undertaken based on surveillance data to protect health, but the key take-home message is that the true value of surveillance data lies in how it is used to inform management actions.

Coordinating a General Surveillance Program for Wildlife Pathogens

The five components of a disease surveillance program must be continually coordinated as it is coordination that allows these independent components to function as a national surveillance program. Coordination of any largescale program can be challenging and can require full-time staff to achieve. These staff are responsible for ensuring that the components of the program operate together to achieve the established objectives of the surveillance program.

In nearly all countries, responsibility for wildlife health and disease management is poorly defined, and the responsibility is formally or informally shared, by several different branches of government including wildlife, environment, public health, agriculture, veterinary services, tourism, economics, border services and international relations. Since a single branch, or agency of government rarely has exclusive authority over wildlife disease issues, the responsibility of coordinating wildlife disease surveillance can also be unclear. Wildlife disease surveillance also required a different and very diverse network of people than do disease surveillance programs for humans and domestic animals. Thus, organizational models for the coordination and operation of other disease surveillance programs may not work well for wildlife disease surveillance. For example, wildlife biologists and ecologists are essential participants in wildlife disease surveillance. Government agencies such as veterinary services and public health, which are familiar with their own forms of disease surveillance, may have little experience working with agencies responsible for wildlife and the environment where biological and ecological expertise is to be found. Additionally, non-government organizations, universities and other groups outside of government often are key participants in wildlife disease surveillance and the coordinator must understand and work closely with such groups as well as with government agencies. Given these challenges and variability across countries, there are many ways in which coordination of wildlife disease surveillance can be organized. A few examples are given below:

1. Coordination by one government agency. It is common for a government agency that needs wildlife disease surveillance information, or is responsible for other aspects of animal health, to feel it must or should undertake coordination of the national wildlife disease surveillance program. This can work well, providing the coordinator is given the flexibility required to engage all the different government and non-government groups needed for an effective program.

2. Coordination by a coalition of government agencies which manage the program together through a written agreement. This has the advantage that the surveillance program is not

considered to be owned by a single agency, and other government agencies may be more willing to support the program.

3. Coordination by a non-government organization. This model facilitates the collaboration among government agencies from different ministries and among government and non-government participants in the surveillance program. Resources for the program can be pooled and managed by the coordinator, and the program is carried out under the authority of the participating government agencies, which also play an oversight and governance role.

No matter how or by whom coordination of wildlife disease surveillance is organized in a country, the WOAH focal point for wildlife can play a key role in assuring and facilitating effective coordination.

Summary – general surveillance

In the preceding sections, we defined general surveillance and the five components that are necessary to establish a national general surveillance program for wildlife health. These components included detection of pathogens and diseases, identification of pathogens and diseases, information management, analysis and communication and taking action. These components must be integrated in order for the surveillance program to be successful. A national general surveillance program forms the backbone of any efforts to understand and manage what pathogens exist in a nation's wildlife population and is an essential tool for detecting and responding to novel emerging diseases associated with wild animal pathogens.

Targeted Surveillance Program for Wildlife Pathogens

Targeted wildlife disease surveillance (also called 'active' surveillance) is defined as searching for evidence of one or more particular pathogens (viruses, bacteria, fungi, protozoa) in one or more wild animal host species. In contrast to general wildlife disease surveillance, targeted surveillance programs usually focus on detection of the target pathogen(s) or infection, rather than diseased animals; however, this is not universal and some targeted surveillance programs are specifically designed to exploit information from individuals displaying signs of disease (e.g., chronic wasting disease weighted surveillance). Given the specificity of targeted surveillance, it is not practical to have targeted surveillance programs for every disease or pathogen. Priorities and criteria for the inclusion of pathogens for targeted surveillance vary from country to country and between different regions of the world. But regardless of the target, the surveillance system should generate information that is needed to improve the current understanding of a certain pathogen or infection, where it occurs and does not occur, how the burden within the population and is becoming more or less common, and at what point appropriate management actions can be taken. Most often, the decision to include a pathogen or infection in a targeted wildlife disease surveillance program is based on the importance of the pathogen to public health and human wellbeing, either directly (e.g. zoonotic pathogens) or indirectly (e.g. pathogens that can have important effects on livestock production or trade).

Targeted wildlife disease surveillance programs are usually developed and implemented for one of the following reasons: to demonstrate freedom from a particular pathogen or infection of

concern; or to identify trends/patterns in the distribution and occurrence of the pathogen. With sufficient effort, most targeted disease surveillance programs that aim to demonstrate freedom from infection are also able to detect the presence of a pathogen should it spread to the country or region. In the first case (to demonstrate freedom from infection), if no or few animals test positive, the collected data can provide evidence (within some statistical confidence) that the pathogen is not present above a certain level of prevalence. When a targeted wildlife surveillance program is designed to measure intensity of an infection or identify trends and patterns in the distribution of particular pathogen a variety of metrics that can be used.

Freedom from disease

Establishing freedom from a particular pathogen (e.g., WOAH-notifiable pathogen/disease) within a population/region involves collecting presence/absence data from individual animals composing/inhabiting the population/region. This means each individual or a subsample of individuals involved are tested for the pathogen of interest. If presence (i.e., the pathogen is identified) is established within an individual, then clearly the individuals are not disease-free. If this was the sole purpose of the investigation, no further work need be conducted. However, even the pathogen is absent from tested individuals, it is possible that the pathogen is present but undetected so it may be important to assess the underlying infection probability of the pathogen within the population/region at specified statistical confidence levels. This infection probability (i.e., design prevalence) may be known if the exact sample size (described below) necessary to achieve a desired confidence for a specified design infection rate was collected; however, it is common the number of individuals sampled will fall short or exceed this target sample size. In these cases, a Bayesian statistical framework and the number of individuals sampled may be used to estimate the underlying infection rate and its upper 95% credible bound. The 95% upper credible bound is the value at which there is a 95% probability the true infection rate is below and is generally the value of most interest. It provides an upper threshold value for potential infection rates within a population. A suitable reference describing this technique and how to incorporate auxiliary information can be found WOAH at http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0089843.

Prevalence

Prevalence is one of the most common disease metrics used to measure the intensity of a pathogen/disease. It is the proportion of individuals that are discovered to have a disease/pathogen during surveillance and characterizes how widespread an infection is within a population. It is usually based on a cross-section of the population, and if based on a single sampling event arises from a “snap-shot” in time. However, during surveillance efforts it may be difficult to determine over what length of time the prevalence metric applies because activities may have occurred over an extended time period prior to it being discovered. Prevalence and its associated measure of precision are easy to calculate, and more sophisticated analyses can be conducted to examine risk factors, spatial and temporal relationships.

Incidence

Incidence is a much less used metric in wildlife disease. Incidence is the rate of ***new*** cases within a population for a given time period and represents the risk of infection. One of the main reasons why incidence is rarely employed is because it requires frequent and regular monitoring of the

population to establish the number of new cases that have occurred since the previous monitoring period. This can be quite difficult for wildlife populations that are often able to move in and out of a region and can succumb to a disease without being observed. However, there are instances that it can be employed if the objectives of the investigation require it. For example, frequent monitoring of a small pond for amphibians that have died due to chytridiomycosis. Like prevalence more sophisticated modeling endeavors can be undertaken to elucidate various risk factors and other relationships. The difference between incidence and prevalence is most important when investigating infections that can lead to chronic diseases; the difference is less important when doing surveillance on infections that cause only acute disease.

Regardless of the chosen metric, data collected through targeted surveillance programs are useful for identifying trends and patterns in infection, particularly across years (to look for temporal trends) or across regions (to identify higher and lower risk areas). This information can then be used to inform and enhance sanitary practices for domestic animals, and wildlife management plans and activities.

Components of targeted wildlife disease surveillance

Following the identification of the target pathogen or infection for the surveillance program, the targeted surveillance program requires the same components as a general surveillance program to be successful: management and analysis of the data collected, interpretation and communication of the findings, and taking action. Similarly, both types of surveillance programs rely on having an established network of people and organizations that work together and communicate well. Lastly, targeted surveillance just as required by general surveillance, needs to have a coordinator to make sure the surveillance program is meeting its objectives by ensuring successful functioning and integration of the components.

Summary – targeted surveillance program for wildlife pathogens

In the preceding sections, we defined targeted surveillance, described key metrics associated with this type of surveillance, and highlighted the similarities in necessary components it has with establishing a national general surveillance program for wildlife health. A national targeted surveillance program can be used by a country to understand the current risk of a specific pathogen/disease being present in wildlife populations, measure the intensity of that disease, ascertain the spatial extent, and to develop management or control actions if warranted.

DESIGNING A NATIONAL SURVEILLANCE PROGRAM

We have described different types of surveillance that could be the cornerstone of a national wildlife health program; however, the question then remains of how to select the appropriate type of surveillance and how to design the program to meet specific needs? The intent of this section is to highlight some of the key considerations that should be addressed to choose the type of surveillance program that should be used and determine the design of the chosen program. It is important to do this initial scoping and clearly lay out a suitable design prior to initiation of activities. Failure to address design issues may result in an inefficient surveillance program or, in the worst-case scenario, the inability to meet the objectives of the national wildlife health program.

Objectives

The objectives are the foundation upon which the entire program is built, and clearly stated objectives not only predetermine the necessary activities, but also provide guidance during surveillance when questions arise. Objectives also define whether targeted, general or both types of surveillance should be the focus of the program. Some key questions that can help formulate objectives are:

1. Why am I conducting surveillance?
2. What do I hope to learn from my surveillance program?
3. What data will I need to collect?
4. What stakeholder groups will need to be leveraged to support the program?
5. What infrastructure do I need for the surveillance program to be successful?

The first question forms the foundation of a surveillance program. There are significant implications regarding the type of surveillance and associated study design depending on how this question is answered. Examples of potential surveillance objectives include:

1. Ensure that a WOAH-notifiable pathogen/disease is not present in my country.
2. Determine if there is a newly emerging disease in my country.
3. Monitoring for an established disease in my country.

The second question expands upon the first and helps in creating an appropriate study design. For example, is the intent of the investigation: to learn whether a specific pathogen is present in individual animals within a particular species in a specific geographic region; to estimate the prevalence of a pathogen; to determine potential risk factors; to determine spatial extent, etc. This information will determine how many individuals will need to be sampled, how sampling will be conducted and over what scale. If properly developed the answer to this question, will define the

host populations to sample, geographic extent and metrics that will be used to for the surveillance program.

Understanding the data needs for a surveillance program is important to ensure critical information is captured, and equally important to not collect too much data. It is a common impulse to try to capture too much information because there is a wealth of data that can be recorded. When planning a surveillance program, it is common that planners identify many, many pieces of information that *could* be recorded for each occurrence of wildlife disease detected and may be useful. In practice, however, attempts to collect large amounts of information systematically for each disease occurrence often fail due to the time and effort needed to collect, record, and enter data into spreadsheets or databases. In the end the collection and recording process breaks down, records become partial and incomplete, and some critically important data go unrecorded. Recording of data and entry into computer systems and its curation takes time and effort (resources). Thus, most often, the best practice is to define the minimum amount of information that is needed to achieve the objectives of the surveillance program, and to ensure that at essential data are always recorded and made part of the permanent record for every disease occurrence.

Answering the 4th question is useful to identify groups of individuals that can sample animals or from whom information concerning morbidity or mortality events may need to be solicited, and to whom surveillance results may need to be communicated. Stakeholder involvement is a requisite for collecting samples and monitoring population health because often resources are not available to support adequate personnel for the surveillance program if stakeholder involvement is lacking. Additionally, early involvement of stakeholders can be beneficial to build political support for establishing a surveillance program. Similarly, identifying stakeholders lays the groundwork for developing an effective public communication strategy from which surveillance results can be disseminated.

Finally, identifying the needed infrastructure to support a surveillance program will define the amount and type of resources needed, the required expertise and establish the necessary collaborations to achieve the objectives of the surveillance program. Answering this question also helps to determine if the objectives of the surveillance program are realistic, or if they need to be scaled back to match available resources. It also helps assess whether available personnel will have the skill sets necessary, or if other personnel needed to be hired or outside collaboration built to fill any identified gaps.

Sampling Design

Once clear objectives have been established, the next steps are to determine:

1. Where and from what populations samples will be collected?
2. What is the metric of interest?
3. How many and in what manner should samples be collected and tested?

There are numerous different sampling designs that can be applied depending on the objectives of the surveillance program. We describe many of the important design considerations below. It is worth noting that there are more design aspects to consider when the objectives require a

targeted surveillance system compared to when they require a general surveillance system. This is because the general surveillance involves as many moribund or dead animals as possible; however, some of the following design considerations are applicable for both types of surveillance systems, and we will try to highlight those cases.

Metrics

There are a variety of metrics that can be used to summarize and analyze information collected during targeted surveillance, but the appropriate metric depends on the objectives and type of surveillance to be undertaken. Understanding how the data will be analyzed is necessary to determine other aspects of a study's design. We previously described three of the most common metrics used for summarizing surveillance data.

For general surveillance, commonly only detections of diseases or pathogens are reported; however, freedom from disease metrics or prevalence estimates from collected samples post-disease detection could be calculated. However, detection of a disease/pathogen via general surveillance often will lead to a follow-up targeted surveillance effort in the population or region from which the disease/pathogen was initially detected.

What host species should be sampled

For targeted surveillance, one important design consideration is what species within a pathogen's host range or which of those affected by a disease will be included in the surveillance effort. Identification of the wild animal species to be sampled will inform how animals will be caught or trapped, from where and by whom. Determining an appropriate sample population will require pre-existing knowledge about the target pathogen or infection. The wild species that are competent hosts (i.e., host range) for the pathogen of interest in the country must be known, along with the approximate size of their population(s) and geographic distribution(s). This preliminary knowledge comes, most often, from general wildlife disease surveillance, scientific studies, or expert knowledge.

The choice of host species should obviously align with the objectives; however, there are a few factors to consider that may increase efficiency of surveillance efforts. First, the behavior of the targeted pathogen in the various host species is critical. For instance, given the difficulty of capturing wild animals, if an objective is to determine if a particular disease or pathogen is present in wild animals in a country, you might select a species that is known to develop actual disease (i.e. show clinical symptoms characteristic of the disease caused by the target pathogen) rather than a species that does not develop disease following infection. This will allow for more focused surveillance with a reduced time to disease detection if it is present. However, if the purpose of the targeted surveillance program is to estimate prevalence of an infection for which the wild animal may be a carrier of the pathogen, but may not develop clinical disease, then you may choose to sample both apparently healthy and sick animals.

A second important aspect to consider are logistical factors such as whether some species easier to find and to sample than others. Focusing efforts on these species should help maximize the use of limited resources. In other instances, it may be critical to focus efforts towards on sampling rare or threatened species to understand the impacts of a disease on small populations. Alternatively, if impacts on specific populations are less important than understanding the spatial extent of a disease and a more common surrogate species is available, it may be advisable to

sample this species in lieu of sampling the rare species to avoid any risk associated with capture and sampling, while still gaining information on the extent of the disease.

Another consideration may be the likelihood of the species to transmit a pathogen to human or domestic animals in which case surveillance could be targeted towards species or populations most likely to transmit across these boundaries. In other situations, it might be appropriate to sample domestic animals to determine whether or not a pathogen of wildlife is present or absent from an area. For example, farm dogs have been used to determine whether plague (*Yersinia pestis*) was present in local rodents. The dogs, which hunted the rodents, were easier to catch and handle than the wild rodents themselves. Lastly, it may be useful to focus efforts on species that "sample" a range of other species, particularly for disease detection. This may involve sampling predator or vector species that feed on a variety of other species. Ultimately, the choice of what species or group of species to include will be situational; however, evaluating the biology and epidemiology of the host and pathogen, logistical constraints, and societal influences through the lens of the objectives of the surveillance program, will ensure the surveillance is not only efficient but also effective.

Where should samples be collected

The next consideration is where samples should be collected to inform the general or targeted surveillance program. If a surveillance program is set up to look for pathogens that are known to affect animals in a neighboring jurisdiction, surveillance activities may be focused along the border region. The decision about where to look for a pathogen also depends on the purpose of the surveillance program. If the intent is to detect a zoonotic disease agent of public health importance, surveillance of sentinel wildlife species might be done primarily in urban areas where the greatest numbers of people live. Similarly, if the purpose is to detect a pathogen of importance to domestic animals, then wildlife may be targeted in and around important agricultural areas.

For targeted surveillance, the geographic region to sample will necessarily depend on where the host animals of interest are located and whether or not their location changes over time. This is particularly important when migratory species are the target of the surveillance program. For example, avian influenza surveillance in wild waterfowl in the northern hemisphere is often conducted in the late summer and early fall when the birds congregate together at staging grounds before their southward migration. At this time and place, there are many birds together in a smaller area, so trapping is easier and many of the birds are young of the year and they tend to be more susceptible to infection with avian influenza than older birds.

Once the overall spatial extent that will be included in the surveillance program is established, sub-regions or units within that region must be chosen if the entire extent cannot be surveyed. The first step in sampling geographical units is to decide whether the spatial extent will be treated as a continuous surface or if it will be aggregated (e.g., broken into discrete grid cells) and the method of aggregation (e.g., grid of cells of equal size or polygons describing political boundaries). Aggregation is the most common approach and will be the major focus of our discussion. Generally, these sampling units can be thought of as either one-stage or two-stage sampling designs. First, a sample of geographical units are selected, and subsequently a census (one-stage) or a second sample of individuals (two-stage) is conducted within the selected geographical units. There are many probabilistic designs that can be used. We will describe several common choices focusing on selecting geographical units, but similar procedures can be implemented at the second stage if individual animals cannot be sampled completely within the selected geographical units. Note to implement many of the sampling techniques described

below, the total number of geographical units that will be included in the surveillance program is required. We describe determining that total number in the next section of this manual.

Simple random sample is most common and easiest probabilistic sampling design to implement. It requires that each unit has an equal probability of being included in the surveillance program. If the data are aggregated this can be accomplished by assigning each grid cell an equal probability of being selected. A random sample can then be drawn using many software programs. If the data are treated as continuous, "n" random points must be generated within the spatial extent. There are several specialized software packages available to easily facilitate selection of points including the statistical program R (https://www.r-project.org/) as well as many different Geographic Information Systems software packages.

Another common type of sampling is stratified random sampling. A stratified random sample is used when a random sample is desired, but there are various non-overlapping strata within the spatial extent that are important and, therefore, must be included in the surveillance program. To employ this technique, independent random samples of equal size are drawn from each stratum using the procedures described above. Thus, the total number of geographical units that will be monitored during surveillance is evenly divided between all the strata of interest. For example, suppose we have human-developed and undeveloped strata within our spatial extent, and the human-developed regions cover a significantly smaller geographic area. We may be interested in assessing differences in the intensity of the outbreak between these strata. In this case, stratified random sampling is a suitable sampling design. It is important to note that there are several different possible means to allocate samples size between strata beyond equal sample sizes. A statistician should be consulted to assure optimal sampling designs are implemented.

In the previous sampling approaches, the probability of a geographical unit or location being selected has been equal across all units or locations. However, unequal probability sampling designs, with varying probabilities of selection, may also be appropriate and are often desirable in some situations. Unequal probability sampling is used when there is auxiliary information available that needs to be considered. This information can then be used to improve (i.e., decrease variability) our estimates of disease metrics, or increase our likelihood of detecting a pathogen if it is present within our spatial extent. For example, suppose we believe that the probability of detecting a pathogen is related to the density of the host on the landscape. Additionally, we have spatially-varying, host density estimates across our spatial extent of interest. If we have aggregated our spatial extent, we can estimate the density for each cell. We can then use the cumulative-size method to estimate the sampling probability for each grid cell. Generally, statistical software packages such as Program R (https://www.r-project.org/) can easily select units using an unequal sampling probability design. Another common use for unequal sampling probability designs is to use the spatial proximity of a grid cell to the location of a known outbreak site. Thus, grid cells close to the outbreak are sampled with a high probability, and that probability decreases with increasing distance from the site. If the spatial extent of interest is not aggregated, unequal sampling probabilities can be used to generate geographic locations; however, these techniques are more involved, and a statistician should be consulted. Therefore, this topic will not be addressed here.

Details for implementing these three sampling designs were described in the 4th Cycle Training Manual on Wildlife Disease Outbreak Investigations using outbreak investigations as case studies. However, the techniques will apply equally well to selecting geographic units in which conduct surveillance.

The last type of sampling, we will describe is convenience sampling, which is the most common type of sampling, particularly for general surveillance efforts. Unfortunately, despite being the most common means of selecting units to sample for surveillance it is also the least rigorous. Convenience sampling does not rely on a probabilistic underpinning to choose geographical units or locations, rather investigators use their own judgment to decide what grid cells or locations are selected. The decision is generally based on logistical considerations such as ease of access. Although this sampling approach is the easiest to implement, it is problematic for several reasons. First, it is not repeatable, which is a tenet of scientific investigations. Secondly, it can create biased samples that lead to incorrect inferences. Lastly, it can severely limit the inference that can be made from the collected data, unless strong and generally invalid assumptions are made. We do not recommend convenience sampling and suggest a probabilistic sampling scheme be used whenever possible!

Regardless of the type of sampling that is used to select the geographic units to include in the surveillance program, the end result is a list of units within the larger spatial extent of interest that will be the focus of the program. If possible, we suggest including a statistician in the design of the surveillance program. They can provide the necessary technical expertise to ensure the selection of geographic units, the number of units and animals (described below) is conducted in a manner that adequately aligns with the objectives of the program.

Sample Size – How many geographical units should be included

Determining the necessary sample sizes when geographical considerations are incorporated into probabilistic sampling designs can be complex because for a given cost there are trade-offs between the number of geographical units sampled and the number of individuals sampled within a geographical unit (described below). In general, the following information or an approximation will be minimally necessary:

1. Desired precision or confidence for the metric used by the surveillance program.
2. Diagnostic test sensitivity and specificity.
3. Costs associated with sampling geographical units and individual animals.
4. Variability in the metric across geographical units.
5. Variability in the metrics across individual animals within geographical units.

For one-stage sampling designs, tools used for determining sample sizes for individuals from a single-site, as previously described, can be used. Additionally, AusVet at https://epitools.ausvet.com.au/ provides some tools for calculating samples size when the objective is to demonstrate freedom from a disease/pathogen using a two-stage design. The tools are set up for sampling individuals across multiple herds, but they can also be used for sampling geographical units by recognizing geographical units can be used in place of individual herds. However, in general, when using two-stage sampling designs for estimating disease intensity metrics during targeted surveillance, it will be necessary to consult a statistician because the required sample sizes of geographical units and individual animals within units will require calculating variances and will often be a computer optimization problem. Specifically, finding the optimal solution may require writing custom computer code to maximize the precision while minimizing the costs, and will need to be tailored to each outbreak investigation.

Sample Size – How many surveillance samples need to be collected within a geographical unit

This is mainly only a consideration for targeted surveillance system because presumably as many moribund or dead animals as possible are collected from geographical units during general surveillance programs. Thus, within each geographical unit included in a targeted surveillance program, sample size calculations can be used to estimate the amount of sampling effort of individual animals is required to achieve the objectives of the surveillance program. The first step in estimating necessary sample sizes is to clearly define the population of interest. This may be quite simple, for example bats in a hibernaculum, or it may require some "hard-thinking". For example, an investigation of migratory bird mortalities involving several species. Regardless, the importance of this step cannot be over-emphasized because it determines the sampling frame, which provides the context for sample size calculations. For example, if I say, "I want to collect enough samples through my general surveillance program to detect a 1% prevalence of pathogen X with a 95% confidence", but do not provide a definition of the population then this statement has no probabilistic meaning. In other words, this means that the 1% prevalence requirement inherently assumes there is a population to which the prevalence applies, and if the population definition is lacking.the value of 1% has no interpretation. Defining the population also is important in determining the spatial extent of sampling (described below) that needs to be conducted and is necessary when reporting results.

Once the population of interest is established, the number of animals that need to be tested as part of a surveillance program depends on three main factors:

1. How confident you want to be in the estimates generated by the surveillance data. Traditionally, most surveillance programs, regardless of their purpose, aim to achieve 95% or 99% confidence:

    - If the purpose of the surveillance program is to demonstrate freedom from infection and none of the sampled animals test positive, then the confidence level is a measure of how certain you can be that the pathogen is not present in the population.

    - If the purpose of the surveillance program is to estimate pathogen prevalence in the population, then the level of confidence is a measure of how certain you can be that the true prevalence is within the range of the apparent prevalence that you have calculated.

2. The size or an estimate of the size of the population of interest.

    - Most wild populations are fairly large and so the size of the population of interest does not have a large impact on how many animals need to be included in the surveillance program. However, in the case of species at risk or other small populations, occasionally the normally required sample size represents a large proportion of all the animals in the population or even a number greater than the total population. In these situations, the sample size can and should be recalculated in consideration of the small total population (see below).

3. The characteristics of the diagnostic tests used.

    - Diagnostic tests are rarely perfect and may over- or underestimate the number of animals infected or not infected (described below). This is particularly the case for wild animal populations for which there are few validated diagnostic tests. The sensitivity and specificity of diagnostic tests can increase OR decrease the sample size needed.

Using these pieces of information, there are some basic equations that can be used to calculate a sample size for surveillance programs. The equations are a bit different, depending on purpose of the program. For surveillance to detect infection or to demonstrate that the pathogen is not present at or below a specified value the equation below can be used to approximate the needed sample size:

$$n = \left(1 - (1 - \alpha)^{\frac{1}{D}}\right) \times \left(N - \frac{(D-1)}{2}\right),$$

where α = desired level of confidence, *N* = number of animals in the population of interest, *D* = number of infected animals in the population of interest, and n = minimum sample size needed to be 95% confident that the pathogen is present at/or below the specified prevalence (i.e., $\frac{D}{N}$), if no infected animals are observed. Two fundamental properties regarding sample size calculations are:

- The rarer you expect the infection to be, the greater the number of animals that will need to be tested.

- The larger the population of interest, the more (but the smaller the proportion) of animals that will need to be tested.

The above equation assumes perfect sensitivity and specificity of the diagnostic test being used, and therefore does not consider the characteristics of the diagnostic tests used. When diagnostic test characteristics need to be included in sample size calculations, the sample size calculations become more complicated and often are solved numerically. Fortunately, there are a number of available tools that take information on the three factors described above and can be used to determine the necessary sample sizes. One web-based suite of tools is called EpiTools and can be accessed at https://epitools.ausvet.com.au/. This suite of tools is quite of extensive and is a valuable surveillance planning tool when attempting to detect disease.

If a pathogen has already been detected in a population, a surveillance program is often instituted to determine the intensity of the disease in a population. The question then becomes how many animals do you need to test to determine how prevalent the pathogen is in the population of interest? To calculate this, you need to apply different equations; however, the information needed is similar: 1) an estimate of the true prevalence and 2) the level of confidence you want or require. You will also need to decide how closely you want the prevalence rate estimated from your surveillance data to be to the true prevalence. When good information about pathogen prevalence in the population is not available, you may wish to calculate a sample size based on several different but possible prevalence estimates and balance the resulting range of sample sizes with the resources available.

Equations:

a) For 95% confidence: $n = \frac{4 \times P(1-P)}{L^2}$

b) For 99% confidence: $n = \frac{6.6 \times P(1-P)}{L^2}$,

where *P* = estimate of the true pathogen prevalence in the population of interest, and *L* = allowable error, which is a measure of how close you want the apparent prevalence to be to the true prevalence.

In general, the more extreme the prevalence estimate is (i.e. if nearly all of the animals in the population are believed to be infected or virtually no animals are thought to be infected), the smaller the number of samples needed to attain the desired level of confidence. When test characteristics need to be included in the sample size calculations the above equations are insufficient, but again there are programs available online that will calculate sample size needed to estimate prevalence and account for imperfect tests. As an example, the same numbers above have been entered into the prevalence sample size calculation page developed by Ausvet (http://epitools.ausvet.com.au/content.php?page=PrevalenceSS).

If you are doing surveillance in a small population (e.g. rare species), then you may need to correct the sample size estimate for small populations. From the examples above, you first determined (assuming a perfect test) that you needed to test 3458 animals. But what if there were only about 5000 animals in total in the population of interest? There are enough animals but it might be very hard to obtain samples from that many. If the calculated required sample size represents 10% or more of the total population, you can adjust the sample size using the following equation:

$$\frac{1}{n^*} = \frac{1}{n} + \frac{1}{N}$$

where n^* = the corrected sample size, n = the estimated sample size before correction from the above equations or an on-line tool, and N = the population size. Alternatively, you can add information to the online program at Epi Tools and it will account for the small population for you, as well as test performance parameters.

In conclusion, all sample size calculations provide estimates of minimum sample size. It is a good idea to increase the number of samples above the minimum value, even when the characteristics of the diagnostic tests have been accounted for, in case some samples are mishandled, there is a problem at the laboratory or for other unforeseen issues that might arise.

Who to sample within an area or population of interest

The choice regarding which animals to include in a targeted surveillance program depends on the objectives of that program. For example, if the purpose of the targeted surveillance program is to detect the potential arrival of a new pathogen, you may want to target animals that show clinical signs of the pathogen or infection. In this case, you simply want to know if the pathogen is present in the population or not. The subgroup of animals in the population that is showing typical clinical signs is more likely to be infected with the pathogen of interest than is the much larger subgroup of animals that are apparently healthy. By focusing on sick animals, you are effectively increasing the true prevalence in the population of interest, and therefore the laboratory test being used in the surveillance program is better able to predict infection (better positive predictive values). This

idea is the basis of weighted surveillance, which has been used to increase the efficiency of chronic wasting disease detection in North America.

Conversely, if the purpose of the targeted wildlife disease surveillance program is to determine pathogen prevalence for the population, then the sampled animals should be as similar as possible to the whole population (i.e., if the population is 60% female, then ideally 60% of the sampled animals would also be female). It is not easy to get a representative sample of a wild animal population. Ideally, to ensure that the sampled animals are representative of the whole population, a random sampling approach should be used. Unfortunately, truly random sampling is rarely possible in the case of wild animal surveillance. Also, typically there is limited information about the population of interest, including the accurate estimates of the number of animals, sex, age or where they are located. Therefore, the utmost effort should be put forth to obtain as nearly as representative of sample as possibly when the objective surveillance is to estimate the intensity of a disease or pathogen within a population or area of interest; however, it should be acknowledged that bias may exist in metrics arising from a non-representative sample.

Bias

Anytime a sample is taken from a population, there is a possibility that bias will be introduced. A biased sample is one that is systematically different from the population, which essentially means metrics may be higher or lower than they should be. For example, if samples are collected from hunters, the animals that they kill may not be representative of the whole population of interest. Hunters may preferentially select for larger, healthier animals from a population. The samples may be from older animals or be more of one sex than another. If animals are live trapped and samples are obtained, the animals trapped may be different from the overall population in some manner. All these differences introduce bias into the surveillance findings, and it may not be possible to even know which direction of the bias in the results (i.e., are estimates too high or too low). Therefore, acknowledgement of potential biases needs to be considered in the interpretation and communication of the results.

WILDLIFE SAMPLE COLLECTION FOR DIAGNOSTIC TESTING

Material in this section was adapted from:

White, C.L. and Dusek, R.J., 2015, Wildlife specimen collection, preservation, and shipment, in Franson, J.C., Friend, M., Gibbs, S.E.J., and Wild, M.A., eds., Field manual of wildlife diseases: U.S. Geological Survey Techniques and Methods 15–C4, 24 p., http://dx.doi.org/10.3133/tm15c4.

Choosing a Sample Type

The surveillance objective and availability of specimens will both need to be considered when selecting a sample type for diagnostic testing during wildlife surveillance (Table 1). For cause of death determination multiple fresh, intact carcasses of affected animals are typically needed so that tissues can be examined for gross and microscopic lesions as well as collected for diagnostic testing. When the objective of sample collection is to determine the presence of disease agents circulating in apparently healthy animals or when sick animals cannot be captured or euthanized a variety of samples from live animals can be collected. These include blood, hair, feathers, feces, ectoparasites, or samples obtained by swabbing lesions or orifices. Collection of environmental samples (e.g., water, soil, feces) may also be useful when disease agents are caused by (e.g., toxins produced during harmful algal blooms) or persist in the environment (e.g., anthrax).

Selection of individual specimens for collection

During a mortality event investigation, a combination of euthanized sick animals (after clinical signs were observed and recorded) and the freshest available carcasses are ideal specimens for diagnostic evaluation. Examining 2 to 3 sick and dead specimens of each affected species will maximize the ability to detect the presence of multiple diseases which is a common occurrence during a mortality event. The freshest carcasses should be used for diagnostic evaluation as the decomposition process can impede the ability to detect tissue lesions as well as pathogen presence. Animals that have recently died will typically have the following characteristics: eyes are intact (preferably not sunken or cloudy), feathers/hair does not pull out easily, no noticeable smell, and no apparent scavenging. Before animals are euthanized the type of tests that need to be performed should be considered. For example, gunshot or stunning to the head should not be used for diseases such as rabies that need brain samples for testing. Lethal injection of chemicals can also affect diagnostic test results for some pathogens.

Table 1. Common sample types used to detect the presence or exposure of disease-causing agents in wildlife. The Disease Technical Cards for non-WOAH listed diseases provide additional information on the type of samples and testing needed for important wildlife diseases.

Sample type	Uses	Examples	Comments
Intact carcasses	Cause of death/ morbidity determination	Various infectious (viral, bacterial, parasitic) or noninfectious agents (toxic substances)	Allows testing of multiple tissues for multiple pathogens and examination of tissues for gross and microscopic lesions
Blood	Evidence of exposure or previous exposure to various pathogens (i.e., anitbodies) and contaminants (e.g., residues or altered enzyme activity) and presence of blood borne pathogens (e.g., hematozoa)	Morbilliviruses, elephant endotheoliotropic herpevirus, equine influenza Lead, insecticide poisoning, mercury, polychlorinated biphenyls Malaria, leucocytozoonosis, babesiosis	Whether antibodies indicate current infection or previous exposure is disease dependent and sometimes species dependent. Paired testing of individual can sometimes be used to establish infection status.
Swabs	Pathogen presence, shedding	Avian influenza (cloacal and oral pharyngeal/tracheal swabs), Batrachochytrium dendrobatidis (skin swab)	Useful for sampling large numbers of specimens for single pathogen (targeted surveillance); does not indicate whether pathogen is causing disease
Feces	Pathogen shedding, presence of parasites,	Salmonella, Escherichia coli, Cryptosporidium spp., Paratuberculosis Toxoplasmosis gondii, Sarcocystis neurona	Useful for determining presence of pathogen or parasite in population or area when animal capture not feasible. Difficult to pair results with individual animals. Does not indicate whether pathogen is causing disease in the population.

Biosafety During Sample Collection

Even if sampling is being conducted on a non-zoonotic pathogen it is still possible for wildlife to harbor other diseases transmissible to humans. Therefore, every animal should be handled to prevent accidental exposures. Keeping a pre-packed kit with basic sample collection supplies and personal protective equipment will reduce the likelihood of forgetting an essential item in the field (Figure 1). At a minimum, when picking up sick and dead animals, disposable gloves, coveralls (or a change of clothes), and rubber boots are recommended. If field necropsies will be performed, then a properly fitted respirator and eye protection should be considered. When disposable bags are not available, inverted plastic bags can be placed over the hand and used to grab carcasses or samples. A fresh pair of gloves should be used for each specimen if cross contamination is a concern for test results. After removal of gloves hands should be washed thoroughly with soap and water as soon as feasible. Equipment should also be washed and disinfected or bagged for later decontamination or disposal before leaving the site.

An example of a basic sampling kit and personal protective equipment that can be pre-packed in a small bag for use in the field.

Collection of Wildlife Specimens

Collection and preservation of carcasses

Carcass collection should be performed while wearing proper personal protective gear (gloves at minimum) and each animal should be placed individually in a bag or container before leaving the site of the event. When picking up carcasses, care should be taken to avoid spreading infectious fluids from the carcasses into the environment and all field gear should be cleaned and disinfected before leaving the site.

Chilling carcasses as soon as possible after collection will help slow the decomposition process which breaks down tissues and proliferates bacteria that may interfere with detection of pathogenic organisms. Decomposition processes are accelerated at higher temperatures; therefore, it is particularly important during warmer months to use a cooler with ice or ice packs to transport carcasses from the collection site. If tissues need to be examined for gross or microscopic (histology) lesions, carcasses should not be frozen as freezing causes cell membrane rupture and interferes with the interpretation of tissue damage caused by a pathogen. Freezing does not, however, interfere with most tests for pathogen detection including bacterial, viral, and fungal cultures; chemical residues; polymerase chain reaction (PCR) tests; and parasite identification. Therefore, freezing is generally recommended if carcasses cannot be examined within 72 hours of collection.

Collection of tissues

When it is not possible to collect an entire carcass for diagnostic testing, tissue samples from various organs can be removed and preserved for testing. Prior to dissecting an animal, an external evaluation should be performed to note any abnormalities as they may provide initial clues to the cause of the event. The basic supplies and equipment to include in a field kit for tissue collection will vary with the species being sampled and types of laboratory analyses. Having a pre-packed kit with basic sampling supplies stored with carcass-collection kits in the office or vehicle will improve the chances of having everything you need when you need it as mortality events can occur at any time.

Small leak-proof plastic bags are useful and inexpensive tissue specimen containers. Specimen identification should be written directly on the bag with a waterproof marker. If lesions are noted, collect multiple samples from them if possible. Each sample for microbiology and virology should be about 1 gram (g). Approximately 2 g of tissue is usually sufficient for toxicology tests but 5–10 g may be needed if multiple toxicology tests will be performed. For histology, sections should be no more than 1 cm thick to allow for fixative to penetrate the tissue. Tissue samples should include all or portions of the lesion as well as adjacent apparently healthy tissue.

Tissues samples should be promptly chilled or frozen if they cannot be examined within 72 hours. If preservatives are available, tissues for histology can be placed immediately in l0% buffered formalin or 95% ethanol solution (except for amphibians where 70–75% ethanol should be used). If using formalin, special care should be taken to avoid contact with skin or inhalation of vapors. Some pathogens can also be detected by molecular techniques on samples stored in 10% formalin solution (or ethanol); however, the laboratory performing the analysis should be contacted if you are unsure if preservatives will affect the analysis. The volume of preservative

should be approximately 10 times the tissue volume. Storage containers can be glass, plastic, or metal but note that some plastics and metal materials may contain substances that interfere with chemical analysis, so it is prudent to contact the laboratory before collection if these analyses may be needed. After 2 or 3 days in 10% formalin, tissues can be transferred to leak-proof plastic bags that contain enough formalin to keep the tissues wet. Specimen identification numbers can be written in permanent marker or pencil on a paper card that is placed inside the bag with the tissues. The identification information should also be written on the bag itself. Bags should be stored in a manner that prevents tissues from being crushed.

Collection of blood

Individuals collecting blood from live animals should receive training in blood collection techniques and animal restraint to avoid traumatic injuries to the animal and to the individual taking samples. Use of anesthetics may be necessary when the sample procedure will cause more than slight or momentary pain or distress. Blood can be used for a variety of analyses including health screening, pathogen detection or exposure (e.g., antibodies), and contaminants analysis. Diagnostic tests can range from the relatively simple determination of packed cell volume (PCV) for anemia and preparation of blood smears to examine blood parasite presence to the more complex PCR analysis for the detection of pathogen RNA and virus-isolation techniques. While numerous tests are available on various blood components, some of the more common sample types are hematocrit, thin blood films/smears, blood serum, blood plasma, preservation of DNA or RNA of both pathogens and hosts, and whole blood.

The number and types of tests to be performed as well as the size of the animal determines the volume of blood and preservation techniques needed to obtain an accurate result. For birds and mammals, a conservative rule to follow is that the amount of blood drawn at one time from a healthy animal should be ≤1% of its body weight. The blood volume to weight ratio for reptiles is slightly lower and blood draws should be limited to 0.5–0.8% of body weight of healthy animals (Campbell, 1996). Blood collection sites and size of instruments vary by taxa and size animal and can be found in numerous veterinary manuals and the published literature.

The intended use will dictate whether whole blood, plasma, or serum is needed as well as the post-collection handling, processing, and storage method. Tubes and vials that can be centrifuged are most commonly used for storage of blood samples. In some cases, tubes with a serum separator can be used to allow for freezing without transferring the serum to another storage tube once the sample is centrifuged. Various types of tubes can also promote or prevent clotting or provide additional additives needed for specific testing.

Similar to carcass storage, refrigeration or freezing is critical to prevent degradation of the sample either by heat or bacterial growth. Once dry, blood smears generally can be stored at room temperature. Serum and red-blood cells can typically be stored frozen until analysis; < −70 ºC is the preferred temperature if there is an interest in isolating virus or other organisms. Dry blood spots on filter paper strips preferably should be stored frozen, but at least refrigerated until testing. With the correct preservatives for testing, some samples can be stored at room temperature.

Collection of swab samples

Swabs can be used to sample large numbers of dead or live animals for the presence of many types of pathogens. Pathogens shed in the mucosal membranes are often detected through the

use of tracheal (typically used on dead birds), oral pharyngeal, cloacal, or nasal swabs. Swabs of fecal and environmental materials can be used to test for environmental persistence of pathogens or when direct swabs of animals are not available.

The appropriate swab location can be determined by type of pathogen and its mode of transmission (respiratory secretions, feces, etc.). For example, oral swabs may be appropriate for detection of viruses transmitted in respiratory secretions but may not be appropriate for detection of bacteria involved in an oral infection because random swabbing of the oral cavity would likely yield a mixture of common oral and environmental bacteria. Appropriate collection devices, specimen containers, culture media, and storage must be used to ensure optimal recovery of microorganisms (Koneman and others, 1997) during diagnostic testing. Improper collection and storage can lead to false results.

Collection of other common non-invasive specimens

Fecal samples can be used to determine presence of viruses or bacteria, gastrointestinal parasites, nutritional and reproductive status, and stress (Bechert, 2012; Leendertz and others, 2006; Waits and Paetkau, 2005). Urine also has been used to detect pathogens and examine reproductive status, urinary function, and stress in wildlife (Bechert, 2012; Cameron and others, 2008; Leendertz and others, 2006). Collection and storage techniques will depend upon the study application and type of analysis to be performed (Palme and others, 2005).

Collection of amphibians

Amphibians decompose rapidly and usually will have large numbers of bacteria and fungi throughout their body by the time they arrive at a diagnostic laboratory. Therefore, live, sick amphibians are generally considered the best diagnostic specimens from which to obtain meaningful bacterial cultures and most types of fungal cultures. However, it should be kept in mind that not all diagnostic laboratories have protocols and approvals in place to handle live amphibian specimens.

Dead amphibians may be suitable for virus cultures, microscopic examination (histology), and toxicological tests. However, amphibians decompose rapidly and are removed quickly by predators so affected animals typically need to be collected the same day they are found. If possible, about one-half of collected carcasses should be preserved in a fixative (so that tissues can be examined) and the other one-half should be frozen (to allow for pathogen detection). Commonly used fixatives include 10% buffered formalin or 70–75% ethanol. If there are a small number of fresh carcasses swabs from the mouth, vent, skin, and skin abnormalities (lesions) can be collected prior to immersion of the animal in the fixative. Prior to immersing the carcass in fixative, the body cavity should be slit open along the ventral midline to ensure fixation of internal organs. For the first 3 to 4 days of fixation, the volume of fixative to volume of carcasses should be at least 10:1. After 3 to 4 days of fixation, the carcasses may be transferred to a minimal amount of fresh fixative, which prevents drying of the specimen.

Decontamination/Disinfection of Field Equipment

Before leaving an area where carcasses are collected, gloves and outer clothing should be removed and double-bagged. Boots and the outside of plastic bags should be disinfected with a commercial disinfectant or a 10 percent (1 part bleach to 9 parts water) solution of chlorine bleach (sodium hypochlorite). Individual specimens should be labeled and then can be bagged together in a second bag before removing them from the area. These precautions will help protect the people in the field and minimize transmission of disease to unaffected wildlife populations.

It is important to wash and disinfect all field equipment that came into contact with animals and surfaces at the disease-event site (nets, minnow traps, tripods, water-quality instruments) as well as the tires and wheel wells of vehicles and boats used to access the site. A solvent (water) or soap (preferably biodegradable) should be used to clean off chunks of mud and debris or vegetation. A 10 percent solution of household bleach is one of the most commonly used disinfectants because of its effectiveness, availability, and rapid decomposition in the environment. Bleach solutions require a 10-minute contact time with surfaces to complete disinfection.

Disinfection involves use of a chemical to kill microorganisms but since disinfectants cannot penetrate chunks of mud and debris they should be used only after objects have been washed. Objects free of mud and debris should be completely immersed in the disinfectant solution or completely wetted with the solution. Soaps and disinfectants should not be discarded into surface water as many are toxic to amphibians, fish, and invertebrates.

GENERAL PRINCIPLES FOR DIAGNOSTIC TESTING PERFORMED ON WILDLIFE

Interpretation of test results from wildlife specimens can be complicated by the lack of baseline data and validated tests for the pathogen or species of interest. Test validation, which includes determining the sensitivity and specificity of a test, requires time, money and expertise, so when tests are validated, it is often only done for small number of species. Guidelines for assay validation in wildlife are available through Chapter 3.6.7 of the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* 2017. The two scenarios described in that chapter include the process for validation of tests for new pathogens as well as the validation of existing tests for new species.

Some key questions that should be considered prior to initiating diagnostic testing include:

- Has the test been validated in the host species and pathogen of interest?
- What is this test intended to measure (e.g., past exposure, pathogen presence), and does that meet your objectives?
- Given the quality of samples collected how well will this test perform?
- What are the characteristics (e.g., sensitivity and specificity) of the test?
- Is the test readily available within your jurisdiction? If not are there other laboratories that can conduct the test?
- What resources are needed for conducting the test?
- Are there legal or regulatory mandates that require specific tests?

When validated tests for the species of interest are not available, another tactic for selecting an appropriate test is to use those that are unlikely to be significantly affected by the host animal species from which the samples were taken. For example, a test that detects a pathogen directly, such as culture of bacteria from a tissue, is less likely to be affected by the host species than is a test that is based on the response of the host animal to infection, such as a test for antibodies or a test for another immune response (Table 2). The Disease Technical Cards for non-WOAH listed diseases in wildlife can also serve as a useful guide to diagnostics used for several important wildlife diseases.

The emerging fields of genomics and metabonomics are also becoming more commonly applied to wildlife epidemiology (Blanchong et al. 2016). Genomics is the study of an organism's genes including interactions of those genes with each other and the environment and applies the techniques of genetics and molecular biology to genetic mapping and DNA sequencing of sets of genes or the complete genomes of selected organisms. This technology has been used successfully to identify the origin and transmission of recent outbreaks including Ebola virus and avian influenza viruses (Gire et al. 2014, Lam and Pybus 2018). Similarly, metabonomics is measures the complete set of metabolites present in an organism or associated biological samples, and examines changes in these due to perturbations such as infection or disease

(Nicholson and Lindon 2008). Metabonomics is closely related to metabolomics, and although there is some disagreement on the differences between these two fields, metabolomics generally focuses on metabolites created at the cellular or organ level under normal endogenous metabolism; whereas, metabonomics focuses on the effects exogenous factors such as disease and understanding the systemic change through time of these factors on the metabolic profiles of complex multicellular systems. Lastly, transcriptomics is the study of the RNA transcripts of a cell, tissue, organism.

Table 2. Examples of tests that may be used for wildlife surveillance and their likelihood of being affected by host species.

	Less likely to be affected by host animal species	**Intermediate**	**More likely to be affected by host animal species**
Tests for pathogens	Direct identification (e.g., parasites) Culture of bacteria fungi protozoa PCR Immunohistochemistry Chemical analysis (toxicology)		
Tests for antibodies or immune response	Virus neutralisation Blocking (competitive) ELISA		Most standard serology tests (e.g., ELISA) Antigen skin tests (e.g., TB)
Other	Genomics	Brain cholinesterase activity [a]	Metabolomics Transcriptomics

[a] Brain cholinesterase activity can be used to screen for poisoning by organophosphate and carbamate insecticides. However, animal species vary greatly in normal background levels.

Sample Quality

The condition of samples can significantly affect test results. In fact, if carcasses are too autolyzed diagnostic testing may not even be possible. In general, collecting biological samples from moribund animals or recent mortalities provides the greatest amount of choices for diagnostic tests. However, if fresh samples cannot be collected, tests that target the pathogen, particularly molecular-based techniques that do not require a viable agent, may be options. A second consideration is the handling of the sample once it has been obtained. Handling can directly impact sample quality and may impact the usefulness of a diagnostic test. Handling involves many different aspects including temperature, transport medium, length of time from collection to diagnostic testing, and container type.

Test Characteristics

It is essential when selecting a diagnostic test to understand the test characteristics and how well it performs. If a test frequently produces incorrect results, then it may be of limited use for surveillance or outbreak investigations. An assessment of the likelihood of a test producing accurate results can be determined through the test validation process. Test validity includes two components: sensitivity and specificity.

Sensitivity and specificity can be determined through the use of 2x2 tables as illustrated below. The key concept is that a positive diagnostic test result does not always mean that the targeted pathogen is present or that the animal was exposed. Likewise, a negative test results does not always mean that the pathogen is absent.

	TRUE PATHOGEN STATUS	
TEST POSITIVE	True positives (A)	False positives (B)
TEST NEGATIVE	False negatives (C)	True negatives (D)

- Sensitivity is the ability of a test to correctly identify those animals with the disease/pathogen (i.e., true positives).

  Sensitivity (SE) = A/A + C

- Specificity is the ability of a test to correctly identify those animals that do not have the disease/pathogen (i.e., true negatives).

Specificity (SP) = D/D+B

A test with low sensitivity would underestimate the pathogen's true prevalence (many infected animals may be missed by the test). This contrasts with a test with low specificity that would overestimate the pathogen's true prevalence (many non-infected animals test positive). True prevalence may never really be known for a population unless all animals in the population were tested with an assay that is 100% accurate, which is extremely rare for any test, even those used for human health. The good news is that when the test sensitivity and specificity are known, we can calculate apparent prevalence and use this information to estimate the true prevalence.

Apparent prevalence is the number of animals testing positive by a diagnostic test divided by the total number of animals in the sample tested

$$\text{Apparent prevalence (AP)} = \frac{A+B}{A+B+C+D}$$

$$\text{True prevalence (TP)} = \frac{AP + SP - 1}{SE + SP - 1}$$

Not only is it helpful to understand these concepts for test interpretation but they can also be used to guide test selection based on whether it the objective of the testing is to "rule in" or "rule out" a particular agent.

- A highly sensitive test should be used if the primary purpose of diagnostic testing is to "*rule out*" the presence of a specific agent or demonstrate freedom from disease. This is because a highly sensitive test has a low probability that an infected/diseased animal will test negative (low number of false negatives).

- A highly specific test should be used if the primary purpose of diagnostic testing is to "*rule-in*" the presence of a specific agent or disease. This is because a highly specific test has a low probability that a healthy animal would test positive (low number of false positives).

- A reality for wildlife investigations and surveillance is that you may not have a validated test or know the true underlying prevalence. But the concepts above can still be useful. For example, a PCR test for a pathogen is generally more sensitive than a culture-based method so may be the right choice if the priority is to limit the number of false negatives. Multiple testing can also be done serially or in parallel to improve the accuracy of results.

- Serial sampling is done by testing samples multiple times and declaring a positive detection only if all tests detect the agent.

Serial sampling is often conducted for surveillance activities with a cheaper "screening test" with high sensitivity being used first. Samples that test positive are then subsequently re-tested with a more expensive confirmatory test with high specificity. To be declared a positive test result, an animal must have tested positive using the first and the second test.

- Parallel sampling involves testing samples with multiple tests and declaring a positive detection if at least one of the tests returns a positive result.

  To be declared a positive test result when using parallel sampling, an animal must test positive to *either* the first or second test. Parallel sampling is typically used to increase sensitivity but it should be kept in mind that it decreases specificity. Additional information on the relationship of sensitivity and specificity during multiple testing is provided in the 4th Cycle Training Manual for WOAH Wildlife Focal Points.

Test Availability

The availability of a desired diagnostic test is another consideration for test selection. If the preferred test is not readily available, it may be necessary to see if other jurisdictions have the capacity and willingness to partner on diagnostics. Such partnerships provide an excellent opportunity to create collaborations and regional networks of partners that can support each other in conducting wildlife outbreak investigations. Most countries do not currently have the resources to support a diagnostic laboratory exclusively for wild animal diseases. Wildlife disease identification most often will be done in laboratories established for veterinary or medical diagnosis.

- Government Veterinary Diagnostic Laboratories

- Veterinary Faculty/University Diagnostic Laboratories
- Private Veterinary Diagnostic Laboratories
- Government Medical Laboratories
- Private Medical Laboratories
- University Research Laboratories
- Hospital Laboratories
- Military Medical or Veterinary Laboratories

International and regional laboratories such as the WOAH laboratory and collaborating centres network are another potential resource. The WOAH has established particular laboratories, based on their expertise, as WOAH Reference Laboratories for some pathogens.

- WOAH Collaborating Centres: https://www.oie.int/scientific-expertise/collaborating-centres/list-of-centres/
- WOAH Reference Laboratories: https://www.oie.int/scientific-expertise/reference-laboratories/list-of-laboratories/

These laboratories can be invaluable resources for testing for particular pathogens and interpretation of test results. There may be challenges in shipping samples to WOAH Reference Laboratories, including intercountry permitting, that should be investigated to determine if sending samples to these laboratories if a viable option. If these obstacles cannot be overcome, a different diagnostic test may need to be used even if it is less preferred.

Resource Requirements

Resource requirements are an important aspect of selecting a diagnostic test. Establishing the availability of funding for diagnostic testing during an outbreak investigation may limit the choice of diagnostic tests. Some tests may also require specialized equipment, materials (e.g., particular media), or personnel for collection or interpretation and these factors should also be considered before selecting a test.

Legal Mandates

A final consideration is whether there are specific legal mandates associated with testing for a pathogen or agent. For example, within the WOAH Terrestrial and Aquatic Codes, tests are prescribed for specific pathogens "to assure the sanitary safety of international trade in terrestrial animals and aquatic animals, and their products". Thus, to demonstrate an outbreak is not caused by a specific WOAH- listed pathogen these particular tests should be conducted. The terrestrial and aquatic manuals provide further guidance in specific application of tests.

- Disease Infections and Infestations Listed by the WOAH: https://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_diagnostic_tests.htm
- Terrestrial Animal Health Code: https://www.oie.int/en/standard-setting/terrestrial-code/access-online/
- Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2019: https://www.oie.int/standard-setting/terrestrial-manual/access-online/
- Aquatic Animal Health Code: https://www.oie.int/en/standard-setting/aquatic-code/access-online/
- Manual of Diagnostic Tests and Vaccines for Aquatic Animals 2019: https://www.oie.int/standard-setting/aquatic-manual/access-online/

DATA COLLECTION DURING A MORTALITY EVENT

Most wildlife disease outbreak investigations seek to determine answers to questions about who (what population is affected), what (disease etiology), where (geographic extent of the problem), and when (time frame of the event) for a wildlife disease event. Data collected during a morbidity or mortality event is vital to understanding causal drivers of the event. For example, recording the recent occurrence of a weather event such as a hailstorm can be used to understand why the primary diagnosis in a large water bird mortality was blunt trauma. Many diseases, such as botulism or harmful algal blooms, have environmental drivers so recording information (e.g., high temperatures and low rainfall) can speed up and ensure appropriate diagnostic testing.

The data needed to investigate wildlife morbidity and mortality can be broken down into two levels: event level data and individual level data. Individual level data should be collected for each specimen to be submitted for diagnostic testing and associated (by identification numbers or some other system) with the event-level data.

Individual level data (collected for each specimen submitted for testing):

- Species
- Sex and age (if known)
- Collection date
- Collection location
- Event ID (or some other mechanism of associating with event level data)
- Preservation method (e.g., chilled, frozen, formalin)
- Contact information

Event level data:

- Location of event (GPS coordinates or as precise a description as possible)
- Land use and environmental factors (e.g., weather conditions surrounding outbreak)
- Population(s) at risk (i.e., contextual information about species present at the site)
- Estimation of morbidity/mortality onset and end date (condition of carcasses may be useful for assessing these dates)
- Species affected
- Estimated or known number of dead animals by species

- Estimated or known number of sick animals by species
- Clinical signs (for example, unusual behavior or physical appearance)
- Age of affected animals (e.g., juvenile/adult)
- Sex of affected animals
- Clinical signs observed in affected animals
- Contact: person(s) reporting the event and their contact information in case additional information is needed
- Laboratory where diagnostics were performed, and identification number of samples submitted for testing

Best Practices for Data Storage and Management

In order to successfully combine datasets, or even to examine trends in a single dataset, similar information needs to be collected over time. Ideally, there would be a set of universally accepted data fields and definitions for wildlife health. However, since global standards do not currently exist for most data fields, it becomes critically important for each data collector to document how they defined and measured the information in each field. Ideally data fields are defined before the data are collected to ensure that same data are collected from one time point to the next and that the data are collected to the same level of specificity. For example, if the latitude and longitude of a mortality event are collected on some occasions and only the county or province are collected on other occasions then it may not be possible to know if reports of an event represent the same event or multiple events. Some options and considerations for defining the most common fields are also described in detail in the 5th cycle OIE workshop manual. A data dictionary (described at the end of this section) that describes the data in each data field should be stored with the data.

Location of the event

As described in the example above it is important to clarify the scale and accuracy of location information collected. Latitude and longitude provide the most precise estimate of location and can be accessed through most smartphones or GPS- enabled mobile devices. Specifying preferences for formats (e.g., decimal degrees, degrees minutes seconds) and geodetic datums (e.g., WGS 84, NAD 83, ETRS 89) will reduce time needed for conversions after data are collected. Requesting additional information about the location such as the name of the park, body of water, town, or other landmarks may also useful, particularly if the distribution of affected animals spans large spatial scales. If there are issues associated with releasing precise locations (e.g., exact locations of endangered or protected species, farm, or other private lands) locations can be skewed to larger spatial scales (e.g., county/province/state/country) before data are distributed more widely.

Land use and environmental factors

Environmental factors such as sudden weather changes, storms, and drought are potential sources of stress for wildlife that can influence mortality events. Water level changes can concentrate or disperse wildlife population as well as influence the presence of vectors such as mosquitoes. Recording land use (agricultural, industrial, etc.) and other human activities (e.g., recent application of fertilizers or pesticides) surrounding the area can also help guide investigations of the mortality event. Since information could be standardized if there are a limited set of possibilities for the dataset but is most often recorded as a free text field in wildlife health databases or spreadsheets.

Estimation of morbidity/mortality onset and end date

The onset and end dates of the disease event will typically be an estimate as it is unlikely that the first or last incidence of disease was directly observed. Nevertheless, these dates are still critical for establishing temporal cycles of the events and understanding whether the events change temporally over time. The condition of carcasses, proportion of sick vs dead animals, the date a suspected environmental factor occurred (e.g., storm), and the date the site was last visited can all be used to assist in this estimation. Dates can be written in a variety of formats so specifying on both paper submission forms as well as in databases or electronic spreadsheets can ensure that that days, months, or years have not been inverted (e.g., “12” could mean the 12th day of a month, the month of December, or 2012).

Species affected

Species affected typically refers to all species observed dead or sick at a given location during an event. Knowing the species affected particularly in relationship to species present can provide clues into the cause of the event as some diseases have a narrow whereas others have a wide host range. The presence of affected species across multiple taxa may also indicate the involvement of a toxin. Since many species have common names that vary by region or language, using scientific names can help ensure consistency in reporting. Providing dropdown menus for species in databases can further increase quality assurance by preventing errors such as misspellings.

Clinical signs

Clinical signs for many diseases in wildlife are nonspecific signs such as listlessness or nonresponsive to disturbance. Nevertheless, clinical signs may help narrow down the potential causes of the disease and direct diagnostic investigations. As with environmental factors this field is typically recorded as a free-text field in wildlife health databases and spreadsheets unless there are a known list of expected clinical signs that can be specified as a menu of options (i.e., drop down list). Common clinical signs for many of significant wildlife diseases are described in the Disease Technical Cards for non-WOAH listed diseases WOAH.

Age of affected animals

Similar to species affected, the age of affected animals can be used to provide clues about the cause of the event as some disease agents may primarily affect young animals due to age-related disease resistance (e.g., ranavirus, chronic wasting disease). General categories such as juvenile, adult, and aged adult may be a sufficient level of detail needed for this data field, however, if more detailed information is needed (e.g., nestling vs fledgling) then it should be specified on paper or electronic datasheet.

Sex of affected animals

Similar to age of affected animals, recording any observed differences in sex of affected animals can provide clues to direct investigations. Sex differences can be due to behavioral differences such as differences between male and female home ranges or care of young but may nevertheless provide important information in determining the cause of the event.

Number of affected animals

The number of affected individuals typically refers to a count of the number of dead and sick animals for a particular species at a given location during an event. There could be separate fields indicating number sick and number dead for each species if it important to distinguish between the two. A total count of all affected animals can be calculated from numbers reported from individual species. For many locations and vegetation types, actual counts of affected animals may be difficult to perform, so estimates of numbers affected may be more easily obtained. However, since individual researchers vary in their likelihood to over or underestimate, it is important to note in the database and metadata that these numbers are estimates.

Diagnoses

Providing a list of diagnoses can help standardize the preferred diagnostic terminology (e.g., infection with chytrid fungus vs chytridiomycosis) and make partners aware of which diagnoses are of interest for reporting purposes. The WOAH maintains a list of wild animal diseases that are not WOAH-listed but that have been selected for monitoring by the WOAH's Working Group on wildlife diseases because of their importance for wild animals and for early warning purposes to protect human and wildlife health. This list can be found here: http://www.oie.int/wahis_2/public/wahidwild.php/Diseaseinformation/popup/diseaselist. It is also important to ensure that the criteria used to make the diagnoses were consistently applied among animals within the same event and among events over time and space. Case definitions as defined by the WOAH are "a set of criteria used to distinguish a case animal or an epidemiological unit from a non-case". Case definitions typically include a scientifically accepted and clearly defined set of field, gross, histopathologic, laboratory, and epidemiologic criteria used to assign an individual to a specific disease category for reporting purposes and are important for counting and classifying cases of a disease consistently across jurisdictions. Case definitions have been previously established for several diseases affecting wild species that are also of agricultural or human health importance and may impact international trade of animals or their products. Where one exists, the case definition in the specific chapter of the WOAH Terrestrial or Aquatic Code should be used.

Constructing a data dictionary

Data dictionaries allow you to define variables and provide contextual information that cannot be captured in the dataset itself (Table 3). This information should be stored with the data so that it can be properly interpreted by current and future users of the dataset. A data dictionary typically includes:

- Variable name
- Data type (integer, text, etc.)
- How the variable was measured and precision of measurement
- Data units
- Data format
- Minimum and maximum values
- Coded values and their meaning
- Representation of null values
- Other important notes about the data

Table 3. Example data dictionary for several of the key fields described in this section. The units and data type for each field has been specified in the “Data Type” column. A detailed description of each field has been provided in the “Data Definition” column.

	A	B	C
1	**Field Name**	**Data Type**	**Data Definition**
2	Event_Type	Integer	1 = Morbidity/Mortality: Sick or dead animals linked spatially and temporally. Occurrence of single animals is included if there is special interest in the species, the suspected agent, the location, or the time of year (e.g., a solitary species, an endangered species, a possible new pathogen, or a range or temporal expansion for an existing pathogen) 2 = Surveillance: positive detections of a pathogen during active surveillance of healthy live, hunter-killed, or euthanized animals (that were not sick before euthanizing).
3	Start_Date	ISO 8601 YYYYMMDD	Beginning date for event (considering all locations).
4	End_Date	ISO 8601 YYYYMMDD	Ending date for event (considering all locations).
5	Affected	Integer	Total number of individuals affected in event. A count of sick plus dead for a morbidity/mortality event and a count of positives for a surveillance event.
6	Dianosis_id	Integer	ID for event diagnosis. Foreign key link to diagnosis look up table.
7	Species	Integer	Species ID. Foreign key link to species lookup table
8	Population	Integer	Estimate of the total population of this species at this location (population at risk). Use the peak number during the course of the event.
9	Sick	Integer	Actual count of the number of sick or injured animals of this species at this location. Include euthanized animals, if any. Use 0 if known to be 0 (instead of leaving blank). Leave blank if there is no count. Avoid re-count of animals, especially if there are repeated visits to a location to assess wildlife health. Consider whether animals initially observed sick were later counted as dead; if so, only count them as dead. Numbers reported should reflect either a snapshot of morbidity/mortality as observed during a one-time site visit, or a synopsis of the numbers affected over the course of an event (e.g., dead = cumulative dead during multiple site visits and sick = number remaining sick or recovered from being sick at the end of the event).
10	Dead	Integer	Actual count of the number of dead animals of this species at this location. Do NOT include euthanized animals. Use 0 if known to be 0 (instead of leaving blank). Leave blank if there is no count. Avoid re-count of animals, especially if there are repeated visits to a location to assess wildlife health. Consider whether animals initially observed sick were later counted as dead; if so, only count them as dead. Numbers reported should reflect either a snapshot of morbidity/mortality as observed during a one-time site visit, or a synopsis of the numbers affected over the course of an event (e.g., dead = cumulative dead during multiple site visits and sick = number remaining sick or recovered from being sick at the end of the event).

DISEASE MANAGEMENT IN WILD ANIMALS

Disease Management Objectives

The three primary objectives of disease management in wild animals are prevention, control, and eradication. Selecting an objective for management is critical for evaluation of an action's success. Selecting an objective for management should also consider factors such as the presence or absence of disease in an area, availability of methods for management, and available resources. Useful questions to consider before management is undertaken include:

- Why is management being performed (e.g., threatens wild animals, human health, or domestic animals)?
- What tools are available for management?
- Is there public and societal support for management?
- What resources (e.g., funding and personnel) are available for management?
- What would success look like (e.g., complete eradication, reduced prevalence, absence of disease for 5 years)?
- How will success be measured?

Disease **prevention** is defined as excluding or preventing the introduction of a disease into unaffected animals or a population. Disease prevention measures can be applied at the individual, population, or community level. Prevention efforts are typically aimed at restricting or modifying human activities (e.g., trade restrictions) rather than physically barring entry of the agent into a population or community. Prevention can also be achieved through measures such as immunization which can provide directly protection to individuals and indirect protection to populations through herd immunity. Immunizations are a primary method of disease prevention in humans and agricultural animals; however, as we will discuss in greater detail in a following section, immunization of wild animals has numerous challenges beyond vaccine development. Once a disease enters a wild population it is incredibly difficult to control due to complexities such as vaccine delivery and widespread movement of wild populations.

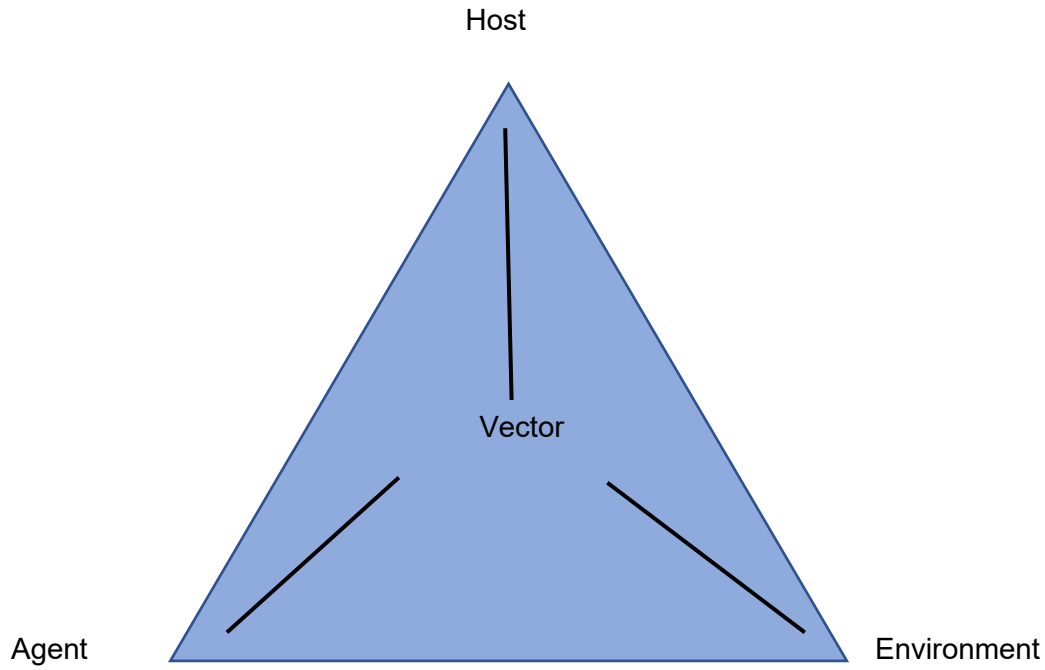
Disease **control** refers to activities designed to reduce the frequency of occurrence or effects of an existing disease within a population to a predetermined level. The level of control is often determined by available resources and level of tolerance for the disease. Available funding often drives the end point for control efforts, as decision makers must assess when the cost of further control outweighs any additional benefit. In this way, control typically results in some level of disease persisting in the population and usually require continued intervention measures (and resources) to maintain the reduction.

Disease **eradication** is the total elimination (i.e., zero incidence) of an existing disease worldwide. The terms eradication and elimination are often used interchangeably but the World Health Organization defines disease eradication as zero incidence of infection caused by an agent worldwide, whereas elimination of an infection or disease refers to zero incidence within a defined

geographic area *(Dowdle 1998*). A key requirement for eradicating a disease is that it must have an effective intervention that can prevent, cure, or otherwise interrupt transmission. To date, only two diseases have been eradicated- smallpox in 1980 and rinderpest in 2011. Smallpox was eradicated through vaccination and rinderpest was eradicated through vaccination and sanitary measures. A global malaria eradication program was established in 1955 but was abandoned in 1969. There is, however, renewed interest in developing a plan for its eradication (see http://endmalaria2040.org/). Eradication of diseases requires significant financial investment and incredible coordination. For example, efforts to eradicate poliomyelitis have cost an estimated $4.5 billion to date. In addition to the financial costs and need for effective treatments, eradication of wildlife diseases are also complicated by characteristics of wild populations themselves including the difficulties of capturing and mass treating animals with free range movement.

Intervention Points

One of the simplest models of disease causation is the epidemiologic triad. It consists of an external **agent**, a susceptible **host**, and an **environment** that brings the host and agent into contact. For some infections, a **vector**, or an organism that carries a pathogen from one host to another, is also part of the disease process. In this model, transmission occurs when an agent leaves its reservoir (which can be a human, animal, or environment), is conveyed by some mode of infection (direct contact, droplet, airborne, vehicle borne, vector borne), and enters a susceptible host to cause disease. We present this model of disease transmission because it is also a useful tool for considering where disease management interventions may be most effective.



Agents can be infectious microorganisms (virus, bacterium, parasite, etc.) or chemical and physical causes of disease and injury.

Prevention and Control – Agents and Vectors

For some diseases the most appropriate intervention is to eliminate its cause. In most cases this type of management is aimed at elimination of the agent from a defined area rather than total its eradication. Control of non-infectious agents (e.g., toxins) often present less technical challenges since management is typically aimed at stopping or reducing the release of a substance into the environment. Since non-infectious agents do not replicate, if a known quantity is released into the environment, it should, theoretically, be possible to physically remove some or all of the agent and prevent additional release. However, some toxins such as polychlorinated biphenyls are very persistent in the environment and cause indirect effects through bioaccumulation in the food chain. Additionally, many toxic agents (or their by-products) are highly useful to humans so controlling these substances may be challenging because of economic or societal costs (Wobeser 1994). In general, substances that have direct, acute effects and have the potential to affect human health (e.g., aquatic mercury poisoning) have had more support for control efforts than those with delayed effects that primarily affect wildlife (e.g., DDT and lead).

For pathogens that persist in the environment steps to minimize contamination of the surrounding area may be needed. For example, the putrefactive processes in unopened carcasses can destroy *Bacillus anthracis* vegetative cells, however, pathogen contamination of the environment contamination can still occur through fluids exiting the nose, mouth, and anus. Since incineration of wildlife carcasses is often not possible during anthrax events, carcasses may be covered with plastic or wetted with 10% formalin to kill external anthrax organisms while preserving an anerobic conditions needed for decomposition (WHO 2008). During avian botulism events, toxin production can increase in decaying carcasses as the bacterium *Clostridium botulinum* prefers nutrient rich anoxic conditions. Maggots feeding on carcasses then concentrate the toxin and infect new hosts when they are consumed. Disposal of carcasses during botulism event may, therefore, help reduce the number of new infections. Disposal of carcasses during mortality events may also be important for minimizing the movement of infectious materials to new locations via scavengers, flies, and rodents.

Common disposal methods for wildlife carcasses include:

- Incineration
- Deep burial
- Landfill
- Composting

Selection of carcass disposal methods should consider their effects on the environment (e.g., air quality and ground water contamination), public health (i.e., potential for disease transmission) and public perception. Carcass disposal should consider country-specific guidelines but general methods have been published by the US Department of Agriculture (*Vantassle and King 2018*) and US Geological Survey (*Friend and Franson 1999a*).

For pathogens that are transmitted by invertebrate vectors, control can be aimed at reduction of vector populations. Although there are numerous highly effective pesticides, many have serious environmental side effects and widespread continued use can exert selective pressure for resistant organisms. For example, endangered black footed ferrets (*Mustela nigripes*) and their

prey, prairie dogs (*Cynomys spp.*), are both highly susceptible to *Yersinia pestis* which is carried by fleas. Control of plague has been attempted through pesticides targeting infected fleas using chemicals such as DDT (*dichloro-diphenyl-trichloroethane*), carbaryl, and permethrins. However, similar to flea control throughout the world (*Rust 2016*), the fleas acquired resistance to the chemicals following continued use (*Barnes 1982*). Other factors affecting the utility of pesticides for wildlife pathogens is that many are broad spectrum and affect non-target invertebrates causing unintended or unknown consequences to ecosystem function. Therefore, use of pesticides to control pathogens may be most useful for small locations and when used in combination with other methods such as environmental control and biological controls (*Wobeser 2004*).

Control can also be aimed at preventing the introduction of pathogens and vectors to new areas and new susceptible hosts. For this type of management methods, should aimed at protecting or supplementing ecological barriers and modifying human behaviors to prevent the translocation of pathogens. Translocation is a common management tool to introduce new species and restore extirpated populations, but there are numerous instances of movement of pathogens with these translocations. For this reason, a number of steps have been developed to reduce pathogen movement during translocations including (*Wobeser 2004*):

- Evaluation of health status of source population including tests for specific diseases-this may also include restrictions on movement of animals from areas where specific diseases are known to occur
- Quarantine of animals to be moved for time period equal to maximum incubation period for diseases of concern
- Diagnostic testing and prophylactic treatment of animals to be moved for diseases of concern

Control – Host Manipulation

Theory

Manipulation of the host(s) population(s) is another major form of disease management for wildlife populations, particularly when there is no intermediate host (snails, vectors, etc). In fact, it may be the most common way managers attempt to control wildlife diseases because techniques for managing populations are often well-developed and familiar to managers. The effectiveness of this type of management lies in first principles of epidemiology. Namely, if exposure to infectious agents is minimized or eliminated the host will be protected from risk of disease. Similarly, if contacts between uninfected and infected hosts can be reduced, disease transmission can likewise be minimized.

The ultimate goal prior to introduction of an infectious agent is to reduce the basic reproduction number, R_0, of the disease. The reproductive number describes the number of new cases arising from a single infected individual in a population comprised entirely of individuals susceptible to the disease in some given unit of time. If R_0 has a value less than 1, an infectious agent cannot invade a population due to the lack of infectious individuals to sustain transmission. Preventative disease control efforts that use manipulation of the host populations try to reduce $R_0 < 1$ by reducing infectious contacts or direct exposure to the infecting agent. After a disease is

established within an area, manipulations of host populations may still be advantageous to reduce the intensity of disease through time.

We demonstrate these ideas using a simple Susceptible-Infected-Recovered (SIR) compartment model without demography for a given population (i.e., 10,000 animals) within a fixed area (i.e., 1,000 km^2). The system of equations for this model are:

$$\frac{dS}{dt} = -\beta SI,$$

$$\frac{dI}{dt} = \beta SI - \gamma I,$$

$$\frac{dR}{dt} = \gamma I,$$

where $\beta =$ the transmission coefficient, $\gamma =$ the recovery rate, and S, I, R are the number of individuals in the susceptible, infected and recovered compartments, respectively. The intent of control efforts aimed at manipulating host populations are generally to influence β. If we define

$$\beta = \kappa \times \frac{N}{A} \times \frac{I}{N} \times \upsilon = \kappa \times \frac{I}{A} \times \upsilon,$$

where N = total populations size, A = area occupied by the population, κ = is a constant such that the contact rate between individuals within a population is proportional to the density, $\frac{N}{A}$, and υ = the probability of pathogen transmission given a contact. It is clear that the rate at which susceptible individuals become infected (i.e., force-of-infection) is the product of the rate of contacts (i.e., $\kappa \times \frac{N}{A}$), the prevalence (i.e., $\frac{I}{N}$) and the probability of successful transmission (i.e., υ). The above equation demonstrates that reducing the contact rate will slow the movement of individuals from the susceptible compartment to the infected compartment. If this reduction is sufficient to reduce the transmission coefficient such that $R_0 = \frac{\kappa \times \upsilon \times N}{A \times \gamma} < 1$, a pathogen will not cause an epidemic. Although this model is quite simple, it demonstrates the underlying theory upon which disease control via manipulation of host populations is based. Examining the equation for the transmission coefficient or R_0, we can see that manipulating the density of animals (i.e., $\frac{N}{A}$) will affect the rate of transmission.

In the Figure 1 we show how the maximum prevalence is reduced and the rate of the epidemic is altered by reducing the density of animals using the above model. Note the figure is presented as the proportion reduction in density (i.e., 0.5 represents 50% reduction in the density) from the starting value of 10 animals/km^2.

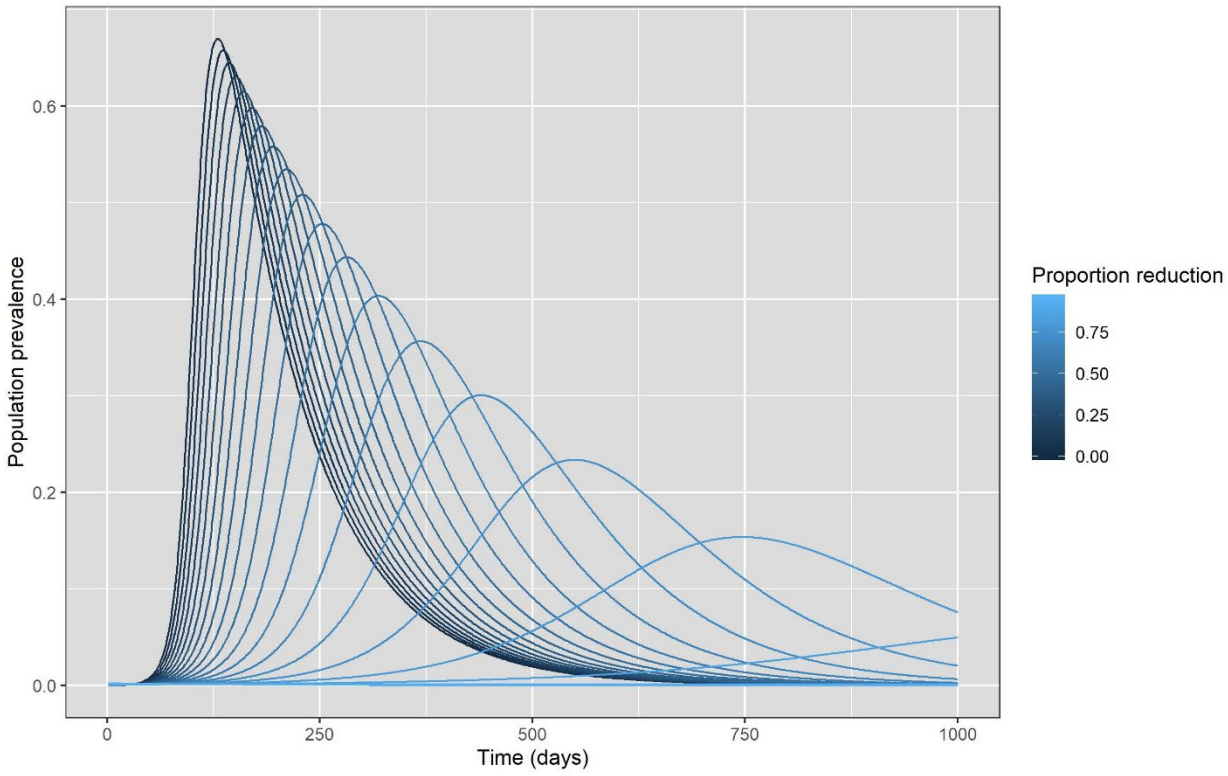


Figure 1 - Demonstration of prevalence curves as the density of the population is reduced.

It is also worth pointing out that the κ and υ terms in these equations are inherent properties of the host and pathogen and therefore are generally not influenced by host manipulation efforts.

We can also use the above model to examine how manipulation of host populations, prior to the introduction of an infectious agent, can reduce the likelihood of an epidemic. In Figure 2 we show how R_0 is influenced by density reduction assuming a starting density of 10 animals/km^2. Examining the figure, we can see that given the parameters from our model, reducing density has a negative linear effect on R_0, but it takes a significant reduction (i.e., > 90% density reduction) to ensure an epidemic does not occur (i.e., $R_0 < 1$). Thus, in this example, a significant effort would be required if preventing an epidemic were the goal of the disease control program. Obviously, these results are not general and are dependent on the parameters specified in our model, but they do illustrate the theoretical underpinnings of many host manipulation efforts. This exercise also shows the utility of leveraging these types of models, particularly when data exists to parameterize them, to guide control efforts and set realistic expectations of expected impacts.

We emphasize understanding the theory behind proposed manipulations of host populations as it is critical to choosing and designing appropriate management actions, as well as communicating these expectations with politicians and the public. Although, it is often the case when dealing with wildlife infectious agents that key information may be lacking to explicitly model a disease as we have done here, nevertheless it may be possible to draw from other similar human, wild or domestic animal diseases that are suspected to behave similarly. This proxy data can then be used to develop an understanding of potential behavior of the disease and its impact on the host populations of interest. Such exercises are also valuable tor organizing existing knowledge and clearly articulating the problem and objectives of management efforts.

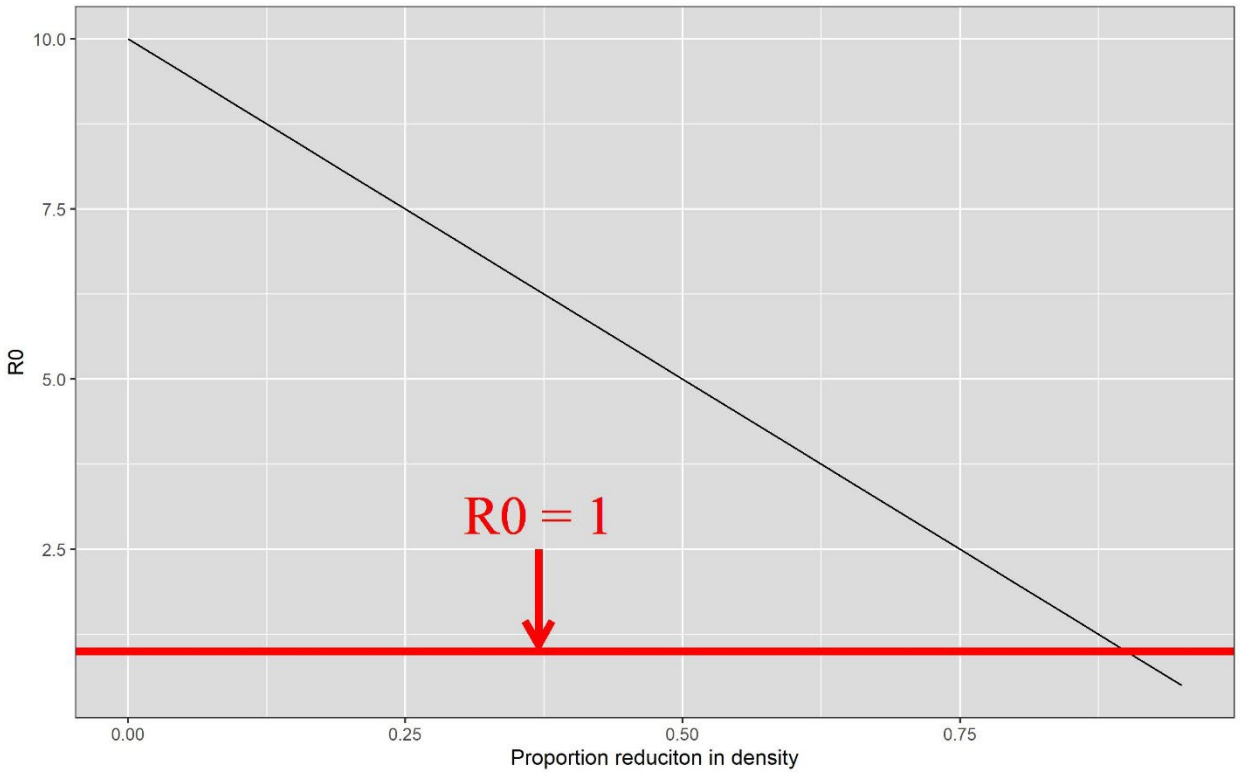


Figure 2 - Demonstration of the impacts of density reduction on R0.

In the next section we will describe major considerations when designing management efforts to manipulate host populations. Our goal is not to detail all potential considerations, which is an impossible task because they will vary across jurisdictions, host populations and infectious agents. Rather our intent is to describe major factors that are essential to account for to maximize the likelihood a disease management program will be successful.

Social Considerations

Although commonly attempted for disease control, manipulation of host populations is a challenging undertaking for a variety of reasons. One of the biggest challenges revolves around the social acceptance of these activities. Public perceptions and expectations around manipulation actions can vary widely within and between jurisdictions and can be influenced by many factors such as economic considerations, anthropogenic uses for populations, lack of biological understanding, deep-seated values or beliefs regarding wild populations, and/or general distrust of governmental institutions. Additionally, public reactions can change rapidly and unexpectedly, potentially due to events outside of the actual disease control efforts. With these swings, undoubtedly the political environment will also evolve, and this can directly impact the efficacy and feasibility of disease control programs. Although it may be impossible to foresee all potential social issues surrounding manipulation of host populations, working to understand the social climate *prior* to attempting these manipulations may help identify the likely public response and any potential pitfalls. This information can then be used to select the appropriate strategy that will maximize public acceptance and, if needed, participation, decreasing the likelihood of failure due to social pressures. It may also delineate various opportunities to build public buy-in for host manipulations. Likewise implementing actions in a transparent manner and establishing a

communication plan that disseminates details of the planned manipulations, the associated importance and need, how success will be measured, and current status of efforts to the public can be a powerful tool to facilitate and maintain public support. However, it is often the case that during control efforts for a rapidly emerging disease there may be little time to for scoping of the social environment. In these cases, it is even more critical that information be communicated in a timely fashion to the affected public because the lack of information can lead to the unintentional or deliberate spread of misinformation and can ultimately lead to the downfall of the management program. Additionally, the level of support from multiple stakeholders about host manipulations can be assessed through formal or informal social surveys, monitoring of popular media, and the hosting of public meetings concurrently with the implementation of an action can help target educational campaigns, as well as illuminate options for the refinement of manipulations to make them more socially acceptable. The take home message is it that manipulation of host populations will undoubtedly fall under public scrutiny, and it is essential to realize that social drivers may be the most influential drivers within the ecosystem of management. To be successful in the long-term, host manipulations must be socially accepted!

System Considerations

The epidemiologic triad is again a helpful image for considering the variety of ways to manipulate host populations to control disease as each component provides basic pieces of information that can help in choosing, designing, and implementing the appropriate management action. It is worth emphasizing again that surveillance is “data for action” so much of the information that is needed to implement disease management can and should be collected during targeted or general wildlife disease surveillance programs.

Agent

The first factor that should be considered is characteristics of the etiological agent. For example, it is critical to determine if the agent is infectious or non-infectious. This will determine if the goal of the population management should be aimed at reducing exposure, contact between conspecifics or both. It will also define the risk posed to human, domestic animals and other wildlife species. Another important characteristic is whether the agent is a recent reintroduction into the system, a re-emerging issue, or occurs more commonly. Systems that have previously been exposed to an agent, particularly, an infectious agent, will likely respond differently to manipulation of host populations than those for which it is a novel introduction. Therefore, the choice and extent of application of management will undoubtedly vary based on the history of the agent in the system. The host range of the agent is another important aspect of planning control efforts. For a non-infectious agent this information delineates the range of species that may be impacted and should be considered during planning. This is also true for infectious agents, but additionally the host range helps identify potential reservoir species, cross-species transmission events that may be significant, and the extent of effort that may be required for control. Similarly, it is useful to know the geographic extent of the disease. Whether a disease is localized of already widely distributed across a region will determine what suite of management actions can be feasibly used. The route of exposure to /transmission of the agent also influences the how host populations may be manipulated. For example, agents that are both directly and indirectly transmitted can be much more difficult to control and may require various manipulations to reduce infection arising from multiple modes of transmission. Even information on the rate of transmission, clinical course of the disease, and any disease-associated mortality is useful to consider when manipulating host populations. These characteristics of the agent determine how

the invasion of a pathogen will impact populations, the timeframe over which disease will be occurring and the risk posed by infected individuals to conspecifics.

These characteristics of the agent are only some of the key pieces of information that can be used to design host manipulation actions. Obviously, the more information that can be brought to bear on the problem, the greater the likelihood the management action will not only be efficient, but also efficacious. If knowledge is limited about the agent, using the information that is available is important. Additionally, if time permits targeted surveillance can be implemented in the population of interest to help acquire additional understanding of the agent.

Host

Attributes of the host population also need to be considered during disease management planning. Involving wildlife biologists and ecologists to help describe the attributes of the individual hosts and their populations is highly recommended because these professionals will often have direct knowledge about the species that will be required to design effective manipulation strategies.

At the population-level, the first attribute to consider, particularly for infectious agents, is the social structure of the population. For example, herd animals may have many potential infectious contacts on a regular basis whereas territorial animals may have only limited contacts with other conspecifics. The social structure will determine the transmission dynamics (e.g., density vs. frequency dependence), how a disease progresses through a population, and suitable management techniques. A second important attribute is the behavior of the population over the course of an annual cycle. Does the host species migrate? Do individuals use their range similarly throughout the year, or does that use pattern change (e.g., use a larger area during the breeding season)? Describing how the hosts use their habitat and the spatial extent of their movements can help elucidate the scale at which population manipulations will need to be applied, the potential costs and benefits ecologically of potential control measures and may suggest times of year when those manipulations should be conducted.

It is also useful to understand potential inter-specific interactions that occur between individuals within a population and other species within the ecosystem. This is particularly true when an infectious agent has an extended host range. The more interactions between susceptible hosts the more difficult the disease will be to control when direct transmission is a major driver of the disease processes. For some of the potential population manipulations, it is also imperative to understand the habitat needs of the host at the individual as well as population scale. Protecting or altering the habitat of the population can be not only used to manipulate population growth and use of an area, but also can be a powerful tool to help populations recover from disease and be resilient to future invasions. Lastly, the overall size or density of a population is valuable information for management planning. The efficacy of a manipulation may be directly tied to the population size and will determine the level of effort that control may require. Additionally, protecting rare or threatened populations may be the highest priority and any manipulation that does not ensure that protection in the long run may be unacceptable.

At the individual host level, the effects of the disease are basic information needs. Does the disease cause clinical signs?

This may well determine if manipulations that target infected individuals can be readily implemented.

What are the endpoints of the disease for the individual; does it result in mortality, immunity, chronic carriage?

These endpoints will determine how aggressive management actions should be, inform forecasts of long-term impact of agents on hosts, the efficacy of various diagnostic tests, and the extent and length of time management may need to be implemented. Also, of interest is whether there may any differential susceptibility among individuals within a population. Individual heterogeneity in response to disease can inform targeted manipulations, but may also hinder management efforts (e.g., a demographic group is asymptomatic but functions as super-spreaders within a population). Likewise, the reproductive rate of a host can directly impact disease processes. It can be difficult to manage disease via population manipulations when a species has a high reproductive rate. In this case, the young may serve as continuous source of susceptible individuals, which can sustain epidemics for long periods of time. It may also be difficult to maintain the effects of population manipulations over time because of the rapid influx of young. Conversely, species with high reproductive rates may recover much more rapidly from disease impacts, and therefore may not warrant extensive management efforts. Lastly, the survival rate is another demographic parameter that can have significant implications for management decisions. The impacts of population manipulations can have vastly different effects on species that are long-lived compared to those that have higher natural mortality. Individual survival directly influences the number of individuals that are susceptible, infected and recovered (using above model descriptions), which translates into varying disease and population dynamics for different survival rates and the efficacy of potential manipulations of host populations. Thus, understanding the demographic processes of the hosts is foundational for developing and implementing disease management actions.

There are other attributes of the host and its populations that may be important in specific systems. But, regardless, understanding the basic biology and ecology of the host is required to be effective at managing disease within host populations via population manipulations. Every effort should be made to collect and collate relevant host information prior to designing any manipulation activity to avoid having unintended or even counter-productive consequences that might inhibit disease control and potentially negatively affect the long-term welfare of the host population.

Environment

We have already described some aspects of the environment that are important consideration when we discussed the habitat needs of the host. However, if we take the environment to have a more holistic meaning, we need to consider the relationship of the host with other components of the ecosystem when deciding on the proper manipulation efforts. For example, manipulating the host population may directly or indirectly impact other important species. These effects may be positive or negative but given the inherently interconnected relationships within an ecosystem there will be undoubtedly consequences beyond those associated with the focal host species. These downstream effects must be included in the decision process when management actions are being chosen to minimize unintended negative repercussions of management. In other words, the need to maintain ecosystem function may limit disease management actions to those that will not severely impact the system's integrity, even if the actions are less efficacious for the particular host population.

The physical attributes of the environment also determine if some population manipulations are feasible. For example, rugged terrain may impede human access and thereby limit the ability to apply management actions to populations inhabiting those regions. Similarly, some environments

may take long periods of time to recover from disturbances, which may reduce viable manipulation strategies to those that minimally impact treatment sites.

The environment is also a critical factor when it serves as a source or reservoir for the etiological agent. In these cases, incorporating the environment into the design of the manipulation action is required reduce reinfection and new infections through this transmission route. There are also some instances when understanding environmental heterogeneity and its effect on the disease process will be key to targeting management efforts towards hosts in the highest risk regions. This can increase the efficacy of population manipulations, and conserve limited resources.

Understanding the role of the environment and the impacts of management at the ecosystem level is one of the most difficult tasks when designing population manipulations. It requires an in-depth knowledge of natural processes at multiple scales. There will likely be significant knowledge gaps that may limit understanding; however, taking the time to map out signigicant system functions and their drivers can highlight potential ecosystem responses to monitor, and can provide the impetus for more in-depth research to address key highlighted deficiencies in understanding.

Logistical considerations

Thus far we have focused our discussion on the system and social considerations surrounding the manipulation of host populations. An equally important consideration are the logistical constraints surrounding proposed management actions. Obviously, the availability of resources to conduct population manipulations is going to determine the type and the extent of manipulations. These resources include not only financial resources, but also personnel. The latter includes not only the appropriate number of personnel, but also those with the correct expertise. The successful implementation of any disease management action requires the skills from a wide variety of disciplines including ecology, epidemiology, statistics, veterinary science, parasitology, pathology, virology, microbiology and data science. This expertise may be leveraged from within or outside of an agency/entity, but success will be an outcome of having the right people engaged in management.

Another logistical consideration is the availability of suitable diagnostic tests (see surveillance section of this workbook for additional details). Some manipulations may explicitly require having a good diagnostic test available (i.e., culling of infected individuals) if they are to be used at all. But, even for those that do not require it for implementation, most manipulations require the availability of a suitable diagnostic test (i.e., ability to distinguish between infected and non-infected animals) to monitor the impacts and ultimate success or failure of a control program. The goal of a control program is generally to reduce the growth and spread of a disease in a population, and only by monitoring the population via targeted surveillance can an assessment be made of the effectiveness of population manipulations.

The last logistical consideration that we describe is assessing whether manipulations can be done passively or need to be done actively from an agency's perspective. For example, if the host species is a game species, it may be possible to manipulate populations through harvest regulations. This would allow population manipulation to be done passively via hunters, which may be a more effective and efficient approach compared to an agency directly manipulating the population. It may also be the case that both active and passive manipulation approaches may need to be implemented for management to be successful. Regardless of the implementation, it is helpful to explore the variety of options that exist for conducting host manipulations.

The logistical considerations are highly variable across jurisdictions, diseases, and system. It is not feasible to capture them all, but they are an important consideration that generally determine how, where and when management is applied. For that reason, we have included them explicitly.

Metrics of Success

Equally important to the aforementioned social and system considerations for designing manipulations of host populations is determining the metrics by which management will be assessed. Defining the metrics of success is often overlooked when designing management strategies. This oversight is likely due to the seemingly obvious purpose of disease control practices (i.e., control disease within a population). Therefore, it seems implicit that success = control of disease. The difficulty arises when we dig deeper and ask the follow-up question what does successful control look like? Delving into that question illuminates that control, unless explicitly defined is an ambiguous concept for wildlife diseases. For example, does control mean complete eradication, a reduction in some intensity metric (prevalence, incidence, frequency, etc.), the prevention of spread or development of new foci, etc. It is essential that how the effectiveness of a manipulation will be assessed be specified prior to the initiation of any activities. This will ensure that correct manipulation to achieve success is applied, and that the requisite information that is needed to assess effectiveness will be collected from the start of control activities. Similarly, defining the goal of a management action in terms to these metrics of success is crucial. This will help determine the distribution of resources, when management can cease or should be abandoned, and is necessary when communicating with politicians or the public. The latter is of particular significance because often the success and availability of resources for population manipulations rests on having continued public and political support. Having clear goals based on defined metrics not only provides scientific validity but also transparency to management actions that may be controversial. In summary, before instituting management aimed at manipulation host populations, delineating how the effects of management will be measured and establishing goals around these metrics is required prior to implementing management.

Host Population Manipulations

Now that we have describe the theory and some major design consideration for conducting manipulations of host populations, we will describe some of the most common manipulations utilized in wildlife species.

Distribution

The first type of host manipulation we will address is the manipulating the distribution of the host population for disease control. The theoretical underpinning of this management action is to reduce the contacts of susceptible individuals with infected conspecifics or reduce exposure to a noninfectious agent. Changing the distribution does not change the overall number of hosts, but rather is aimed at changing the area that those hosts inhabit. Using our simple infectious disease model above, this manipulation essentially changes the *A* parameter, which effectively changes the density. Figure 3 shows the result of doubling the area a population is using on the overall prevalence through time and then introducing a pathogen. Note we are using the same parameters as previously described for the model above.

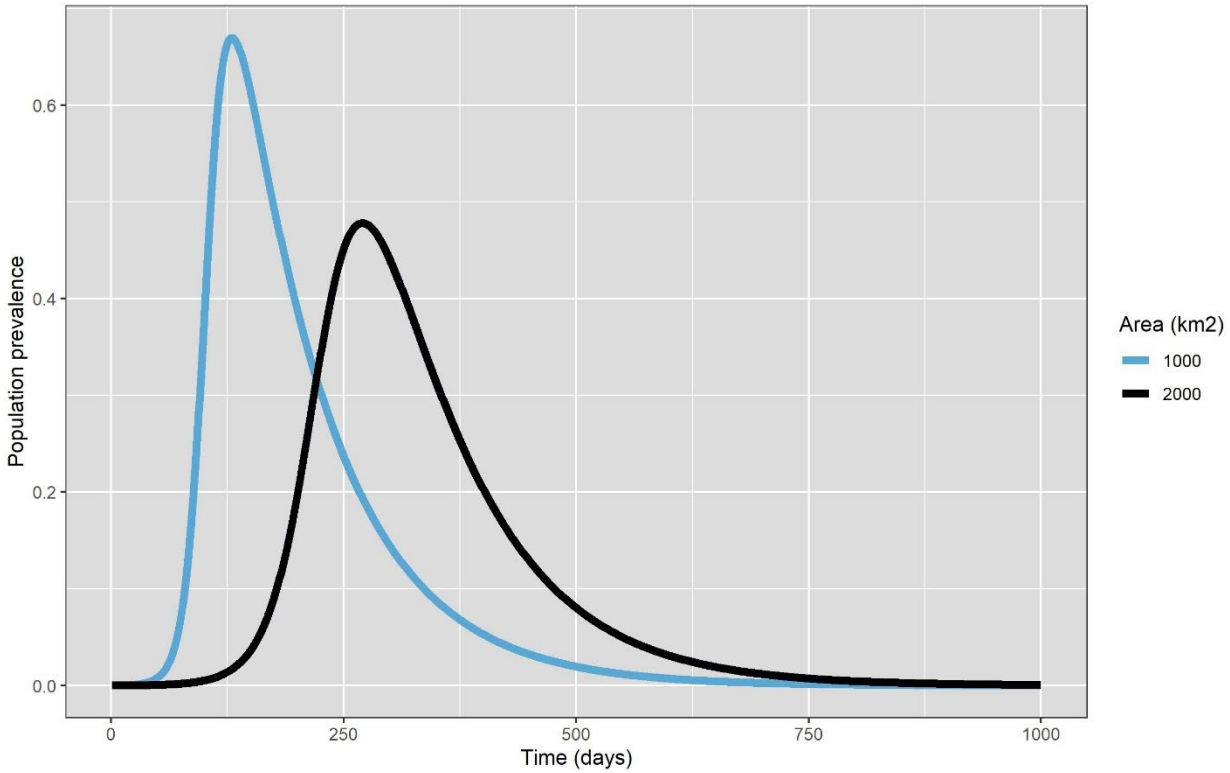


Figure 3-Impacts of increasing the area used by a population on prevalence.

It is evident from this example that doubling the area over which a population is dispersed, reduces the maximum prevalence, total number of infected, and flattens the epidemic curve through time. Therefore, this would be the expected outcomes that manipulating the distribution of hosts may achieve, although exact impacts may vary depending on when the manipulation was implemented relative to the introduction of the disease. Also, in some instances, where there is a point-source of non-infectious agent, or the disease is highly localized altering distributions may have much more dramatic effects.

There are a number of potential ways that the distribution of the hosts can be altered. The first is dispersing or shifting areas used by the host population. This strategy may be recommended particularly when an outbreak or non-infectious agent exposure is localized, and additional unaffected and suitable habitat exists within the region. An example of where dispersal may be recommended is when a site is highly contaminated with lead shot or a wetland complex is experiencing a botulism outbreak (*Friend and Franson 1999b*). In this case, waterfowl and other bird species may be hazed or captured and transplanted to new areas away from these sites. Alternatively, animals may be lured via baiting or habitat improvements away from problem sites. It is important to not underestimate the resources and effort that dispersing or translocating wildlife populations entails, particularly for some species such as bison that are dangerous and difficult to handle or scare (*Wobeser 1994*). Additionally, if dispersal is successful its effects and benefits are likely only transient unless efforts are maintained through time, or habitat is altered to reduce the suitability for the host population (e.g., draining a wetland to discourage use by waterfowl). The latter should be carefully considered within the context of the impacts on nontarget species. Moreover, when deciding to disperse a host population it is imperative that the risk of dispersing an infectious agent be considered. If the disease is emerging and restricted in extent, dispersing individuals may be counter-productive and hinder disease control efforts. In these cases, it may

be advisable to depopulate the local area to protect the greater population of the host (*Wobeser 1994*). Conversely, if the disease or pathogen is known to occur widely and other factors such as environmental characteristics drive emergence, dispersal may be a viable management option.

It is also important to consider other impacts of dispersal such as increased crop depredations or wildlife-livestock interactions that may result from dispersal efforts. For example, a complex of elk feedgrounds in Wyoming, USA was established to provide alternative nutritional sources during the winter for declining elk (*Cervus canadensis*) herds and to reduce conflict with agricultural and livestock interests. Elk herds have since rebounded and large number of elk now congregate on the feedgrounds each winter. Of particular concern is the occurrence of Brucellosis (*Brucella abortis*) in these elk herds as well as bison herds in the surrounding region. This presents a difficult dilemma for disease managers because the feedgrounds functionally help keep livestock and elk separated; however, the large congregations of individuals create increased risk of infection. More recently Chronic Wasting Disease (CWD) poses a threat to these herds with artificially high concentration increasing risks (*Cotterill et al. 2018*). This example highlights both the benefits and risks of influencing animal distributions through intentional distributional changes. In short, dispersing or shifting use areas can be an effective tool for managing animal distributions to achieve disease control objectives, but the direct and indirect effects must be carefully weighed.

Another management option that can be employed to influence host distributions is forced separation of infected and uninfected individuals or prevention of contact with a non-infectious agent. When fencing is considered as a management option, it is generally intended to reduce the spread of infected hosts into regions or to reduce transmission within already affected areas by altering host distributions. Most examples where fencing has been demonstrated to be successful involve separation of wildlife and livestock. For example, extensive fencing has been utilized to reduce incidence of Foot and Mouth Disease (FMD) and t*rypanosomiasis* in livestock, particularly, in Botswana, Zimbabwe, Namibia and South Africa (*Thomson et al. 2013*); however, the effectiveness of fencing and other control measures has declined through time. Fencing has also been used to protect healthy reintroduced populations of the Tasmanian devil in Australia from individuals infected with Devil Facial Tumor Disease (*DFTD; Woods et al. 2018*). Despite these examples, it is rare to find strong evidence of the effectiveness of fencing for control of diseases that are solely affecting wildlife, particularly, if the intention it to separate infected and uninfected wildlife *(Mysterud and Rolandsen 2019*). Fencing is most likely to be an effective wildlife disease management tool when used to prevent access to sites/point sources known to be contaminated with infectious or non-infectious agents (*Mysterud and Rolandsen 2019, Wobeser 1994*).

There are multiple considerations surrounding the use of fencing that effect the success of this management tool (*Mysterud and Rolandsen 2019*). For example, if the fence is intended to separate infected and uninfected individual a rigorous targeted surveillance program will be needed to inform placement of the fence. The behavioral characteristics of the host must be factored into fencing designs as well. The leaping ability, digging ability, tendency to challenge the fence, swimming ability, etc. will determine the height, placement, need for underground construction or use of electricity in fencing design. The type and characteristics of agent are also essential to understand. If an infectious agent is transmitted via vectors, fencing may be ineffective. Similarly, if it is directly transmitted it may require double fencing to minimize potential transmission via fence-line contacts. This can double the cost of construction and maintenance. Agents that affect multiple hosts may also pose difficult challenges because the fence must then be designed to change the movement patterns of all potential hosts. This may be unrealistic if the pathogen's host range include species of small size or those that can fly.

The disease state of the system also defines the efficacy and design of fencing. Within endemic disease areas, fencing may be effective if it can exclude hosts from "hot spots" of infection (e.g., mineral licks, infected food sources, etc.; Mysterud and Rolandsen 2019) and thereby reduce the rate of indirect transmission. But when trying to actively separate infected from uninfected individuals during a disease outbreak, fencing may be less effective for disease control.

In addition to correctly designing and placing the fence, the continued maintenance of the fencing can be difficult. Maintenance fencing will be an on-going effort, and the associated cost in resources in personnel is not trivial, particularly where other wildlife species (e.g., elephants) or other natural events (e.g., windstorms) present an ever-present risk of breaching the fence in addition to routine structural failure. A single breaching event may cause the passage of infected individuals, and the complete failure of the entire disease control effort (*Mysterud and Rolandsen 2019*). Lastly, the role of human-assisted movements of the pathogen or host or movements due to fence breaks where human infrastructure is located (i.e., roads) must be accounted for in deciding if fencing is a viable option and in any subsequent design. *(Bode and Wintle 2010*) provide a simple framework for designing fencing that may be useful during planning phases.

Despite having positive effects, fencing can have profound and unintended negative consequences including extensive habitat destruction during construction, the disruption of migratory pathways of many wildlife species, the limitation of gene flow between populations, the destruction of social networks, creation of population sinks, and direct mortality. It is important to remember that disease control efforts including fencing are not done in a vacuum, and decision makers must weigh both direct and indirect impacts throughout the system when choosing the most appropriate disease control strategy.

In conclusion there are different methods that can be used to manipulate the distribution of host populations. These management options have the benefit of not requiring direct reduction of the size of the population; however, their implementation may be challenging, have unintended side-effects, and the longevity of their impacts may be short-lived. Table 4 at the end of this workbook provides a simple tool to organize knowledge and help in assessing whether changing host distribution is likely to be successful for controlling disease.

Selective Removal

Another potential management tool for manipulation of host populations is selective removal of individuals from the host population. The usual application of this tool involves culling infected individuals from the population to reduce the risk of transmission of infectious agent. It essentially operates on the same epidemiological principle as quarantining individuals in the human health realm *(Wobeser 1994*) as it is used reduce contact between healthy individuals and sick conspecifics. Considering the model presented at the beginning of this section, the goal of selective removal of infected animals is to reduce the transmission coefficient β, and this goal is achieved by reducing the prevalence in the population. The potential impact that selective removal can on disease processes is illustrated in Figure 4 where we begin culling after day 75 of the outbreak and show the impacts for various daily rates of removal of infected individuals (i.e., 0.01 = 1% of the infected individuals were removed each day). Examination of this figure demonstrates the dramatic impact that selective removal of infected can have on the course of disease in a population.

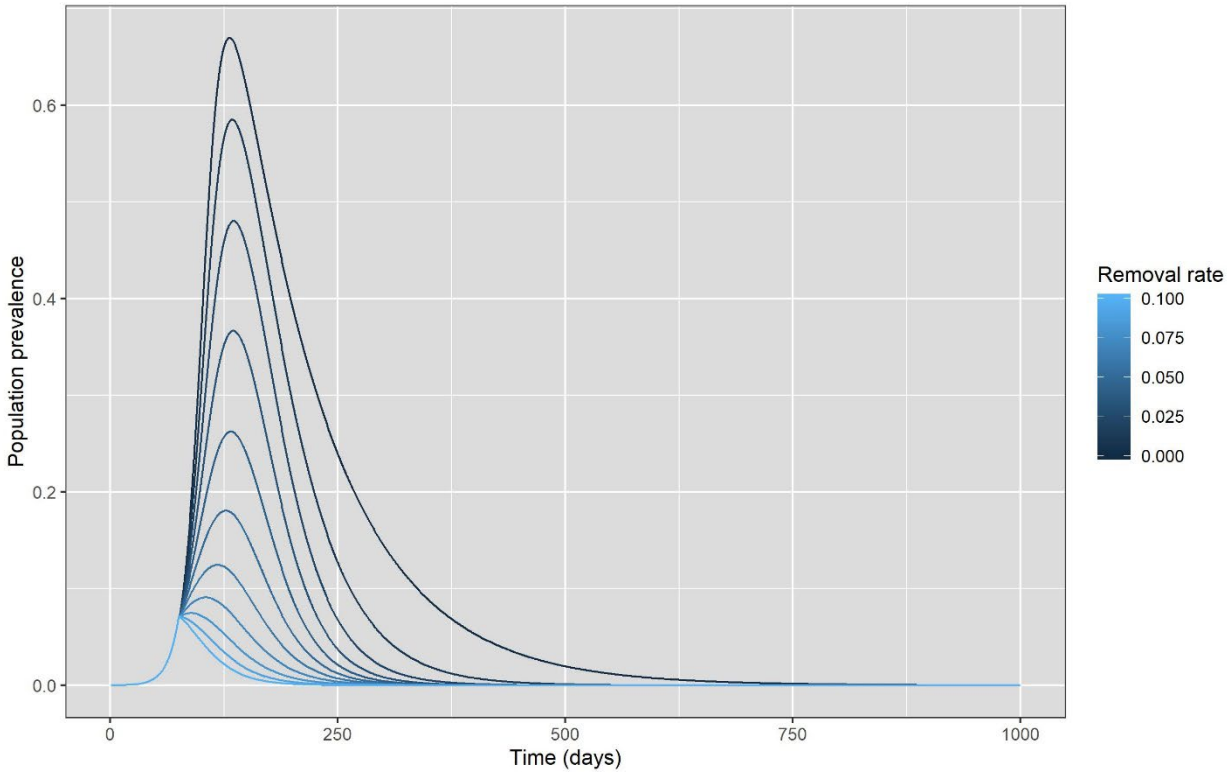


Figure 4-Impact of selective removal of infected individuals on prevalence in the host population.

Although selective removal can be highly impactful, to utilize this disease control practice the appropriate conditions must exist. First, there must be an adequate means to identify infected individuals. If the disease presents with easily discernible clinical signs, these may be sufficient to allow for the removal of infected individuals. However, if clinical signs are not readily apparent or if they present late in the course of the disease, it will be important to have a trust-worthy diagnostic test available to detect infected individuals. In this situation it will also be necessary to be able to associate test results with individual animals so that infected animals can be removed (i.e., test and cull). Ideally, all animals in a population would be tested and untested animals would be separated from known negative animals until all infected animals can be removed. In practice, this will be impractical for most wildlife disease situations. It is more likely that a sustained test and cull program will be required such that there is a continuous reduction of infected individuals to sufficiently slow or stop the epidemic.

Selective removal of infected individuals has been used to successfully reduce prevalence of bovine tuberculosis (*Mycobacterium bovis*) in African buffalo (*Syncerus caffer*) in South Africa (le Roex et al. 2016). However, testing and culling of bison (Bison bison) has not appeared to be effective in controlling brucellosis (*Brucella abortus*) in free-ranging wildlife in Yellowstone National Park in the United States (*Bienen and Tabor, 2006*). Similarly, selective culling was not successful in slowing infection or reducing the impact of DFTD in Tasmanian devils (Lachish et al. 2010). The lack of success of this effort was attributed to the pattern of contacts within the host population (i.e., frequency-dependent), the long latent period and high degree of infectivity of the disease, and the presence of a cryptic hidden disease reservoir or continual immigration of diseased individuals. Therefore, as appealing at this method may seem, the efficacy of selective removal of infected individuals varies according to system characteristics.

Selective removal can also be focused on removing only key infected individuals that disproportionately drive disease incidence in the populations (i.e., super spreaders). For example, in North America bighorn sheep populations have been significantly affected by a respiratory disease caused by *Mycoplasma ovipneumoniae (Movi)*. All-age epizootics can cause significant declines in population size as well as the inability to recruit lambs. In affected populations, lambs are born each year but most succumb to respiratory disease at 6—11 weeks of age preventing the recovery of the population. Recently, intensive epidemiological investigations have demonstrated individual heterogeneity in *Movi* shedding is likely a key driver of disease dynamics with a few chronically infected individuals driving transmission throughout the population (*Plowright et al. 2017*). By removing these chronically infected individuals (as determined through repeated testing), from a free-ranging bighorn population disease was reduced in adults and lamb survival improved (*Garwood et al. 2020*). Thus, for some disease systems, selective removal of chronically infected individuals may be a management option to improve herd health and can reduce the number of animals that need to be removed as compared to selective removal of all infected individuals.

Although less commonly considered, selective removal can also be used to reduce the only those subpopulations or demographic groups most at risk of becoming infected or transmitting a pathogen within a host population. By focusing removal on these groups within a population, the overall risk of disease introduction or transmission can be decreased. This approach has been used in managing Newcastle disease in double-crested cormorants (*Phalacrocorax auratus*). Newcastle disease is OI-reportable disease that causes acute disease in poultry and occurs in wild populations of double-crested cormorants in the Midwestern United States (*White et al. 2015*). However, the disease primarily causes clinical signs in juvenile birds with adults being largely asymptomatic. To control populations of cormorants as well as lessen risk of Newcastle disease, egg oiling is used to selectively reduce hatching and recruitment of susceptible juveniles into the population (White et al. 2015). The effectiveness of egg oiling in controlling Newcastle disease outbreaks has not been critically evaluated; however, the practice provides an example of targeting a specific population demographic for selective removal.

Although there are many factors to consider before choosing to implement selective removal in a population, one of the most important is the social acceptance or tolerance of these techniques. Although often more accepted than random culling for density reduction (described in the next section), there may still be social resistance to lethal disease control options. It is critical to have clearly stated goals, well-developed assessment methods with clearly defined metrics of success, and a communication plan that emphasizes timely dissemination of program results to the public to ensure transparency around control efforts. This will help to build and maintain public support for selective removal. Also, public outreach campaigns prior to the initiation of removal can be informative to highlight potential social pitfalls that can interfere with control efforts and discover refinements to procedures that may increase public support for selective removal.

Lastly, the use of selective removal requires a multi-disciplinary approach to be successful. The influence of the ecology of the host and agent on the disease processes cannot be ignored when implementing this control strategy. Failing to integrate scientists with ecological knowledge into management design can result in a lowered efficacy of control options or even counter-intuitive or detrimental results owing to unexpected population or community responses. Similarly, mathematical modeling of the outbreak may be particularly useful for determining if test and cull methods are likely to achieve the desired results, within a reasonable timeframe, given the real-world constraints of the system. Incorporating social scientists into planning is also advisable to help understand the social environment in which management will be applied, and to help devise methods to ensure support for control efforts. The fact is that manipulating host populations is

altering a complex and inter-woven system of ecological players and processes, and although a complete understanding of the totality of effects of perturbations on this system can never be understood *a priori*; nevertheless, bringing together scientists from these disparate disciplines greatly increases the chances of forecasting the most relevant impacts of management on the system.

In conclusion, selective removal of individuals from the population can be an important disease control tool. It provides a more precise application of management than general culling or density of reduction, as described below. In some systems, it may achieve disease management objectives more quickly and cost-effectively; however, its suitability needs to be carefully evaluated for each system to which its application is proposed.

Density Reduction

The last management tool that we will discuss for manipulating host populations is perhaps the one most commonly applied for wildlife disease management—reduction in the density of the host population. The theoretical underpinning of this approach is that the transmission, coefficient β, for the agent arises from a density-dependent process. Using the simple disease model again from the beginning of this section, Figure 5 shows the impacts on the disease process of an on-going program of density reduction that begins after day 75 of an outbreak.

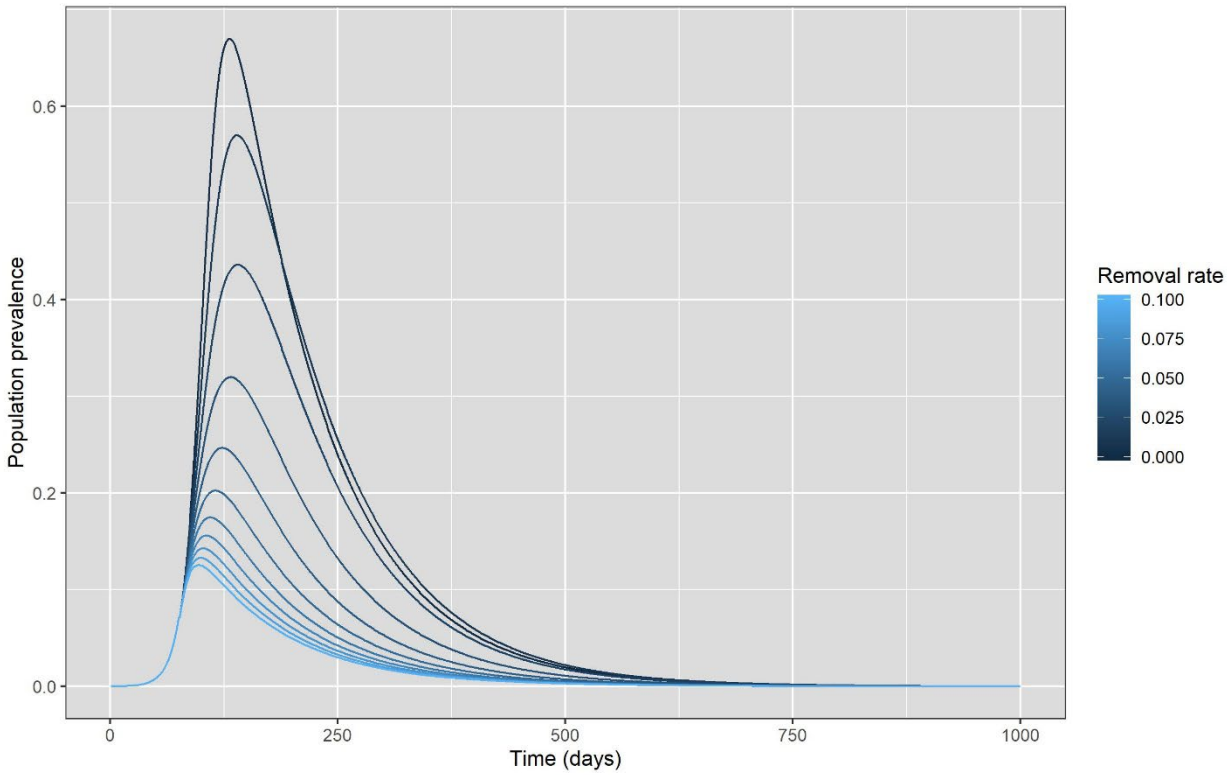


Figure 5 - Impact of continuous density reduction on prevalence.

Although this figure demonstrates that sufficient density reduction can alter the course of disease, there are several important aspects of these results that warrant further attention. First, they assume that animals are randomly selected for removal independent of disease state. This means that recovered individuals are removed at the same rate as the susceptible and infected

individuals. This results in needless removal of animals. Also, we have assumed a constant rate of removal, which may be difficult to maintain, and equally problematic is that sustained removal results in population sizes that can become extremely low. Obviously, this would be undesirable in real-world disease control scenarios.

We can also relax the assumption of continual removal, and instead reduce the population size at one time point. Figure 6 illustrates the effect of density reduction on day 76, on disease prevalence given reduction efforts of various sizes. It is apparent that to have a significant impact on the prevalence a large proportion of the population must be removed (i.e., > 80%), and the effects are much less dramatic than those for continuous removal. However, a onetime effort to reduce population may be more feasible in some disease systems than a continuous removal program.

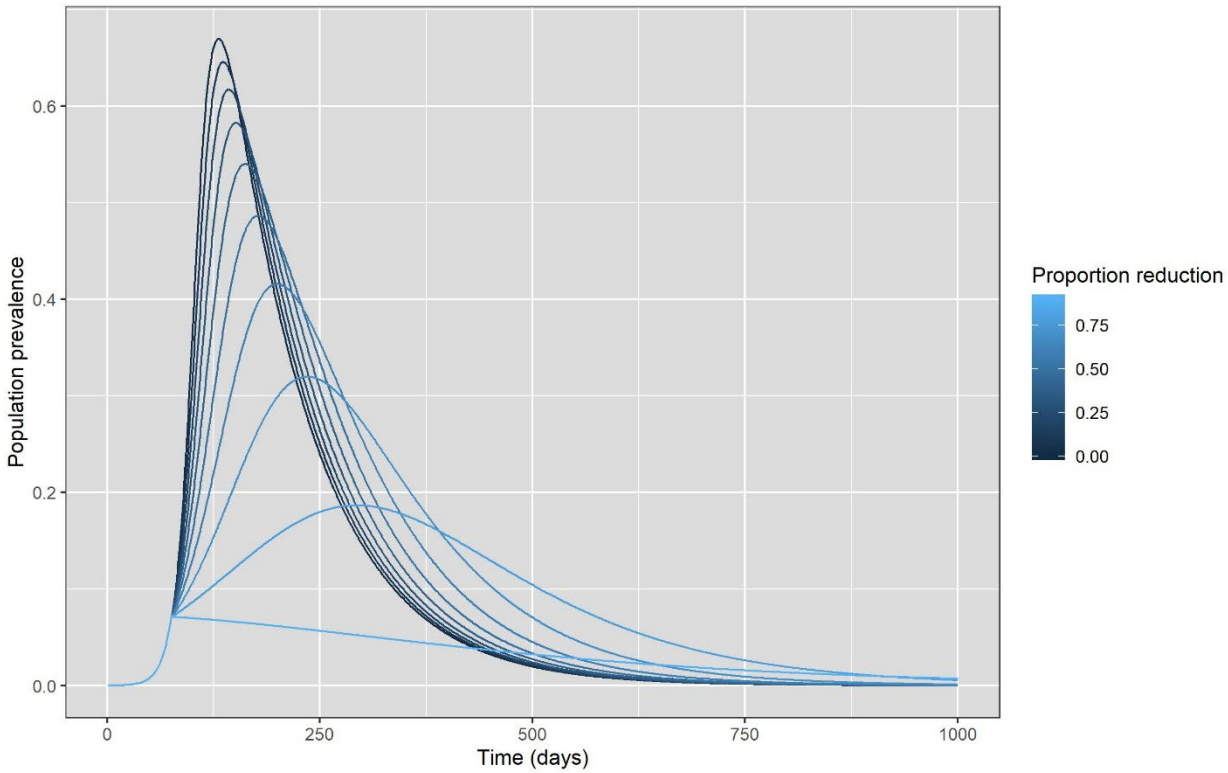


Figure 6-Impact of a onetime population reduction on prevalence.

The above examples focus on removal after disease has been occurring within the population for 75 days. But often density reduction is implemented prior to disease introduction. In these instances, the goal is to reduce R_0 for a disease within a population such that the epidemic cannot become established in the population should it be exposed to the infectious agent. Figure 2 in the Theory section illustrates how values of R_0 vary for our example model as the population is reduced. In our case, the reduction in population must exceed 90% before R_0 falls below a value of 1.

From these simple examples, it is clear density reduction can have highly variable effects on the transmission process depending on the system, as well as, when and how it is applied. Therefore, although commonly used for wildlife disease, density reduction needs to be carefully planned to

ensure it is the correct management option to achieve control objectives and that it is conducted properly to maximize the chance of success.

To that end, there are numerous considerations associated with design of density reductions. The first is how reduction will be conducted. Density reduction is most commonly done through human-mediated, lethal methods, but for some species this is not appropriate. In the case of species of conservation concern, individuals are of high value and so trap and translocate operations may be feasible to achieve the necessary reduction. Additionally, in some systems protecting or promoting predator populations may be a feasible means to achieve lower densities, particularly, if used in concert with human-mediated methods. Habitat manipulations can also be used to reduce densities. These can be conducted through extensive environmental manipulations, or by changing human behaviors and use of the landscape. For example, the discontinuation of supplemental feeding of wildlife may reduce unnatural concentrations of animals and may also affect survival and reproduction of individuals.

The second consideration is the scale at which the reduction will be applied. Density might be reduced at a local site, over a larger area perhaps in a buffer around a newly discovered disease foci or may occur over an expansive area (Wobeser 1994). Determining the necessary scale will generally require information from a targeted surveillance program that can assess the spatial extent of the disease on the landscape. The larger the required scale, the more difficult and resource intensive reduction efforts will be, and the less likely they will be successful (Wobeser 1994). Another consideration to assess is if the disease is novel to the system or endemic. In the latter case, it is also important to understand the intensity of the infection. Populations in which diseases are endemic are much less likely to amenable to disease control through reduction in density because of potentially higher levels and spatial extent of infection within the population. Thus, reduction in density is going to be most effective for prevention of entry of disease (i.e., lowering R_0), but will likely be less effective where disease is established. It is worth noting that in the cases where disease is well-established density reduction may still be of use, particularly, when its purpose is to prevent disease spread to other adjacent populations.

The characteristics of the pathogen are also determinants of whether density reduction is a viable option. The mode of transmission can affect the needed extent of disease control efforts. For example, density reduction will be more likely to succeed if the disease is directly transmitted and will be less successful when transmission involves an environmental reservoir or is spread via vectors. The transmission process will also determine if density reduction will even be successful. If the transmission follows a frequency dependent process, density reduction will fail to control the disease, and if rate of spread of the infectious agent is rapid, there may not to be suitable time to design and implement density reduction programs before such efforts will be futile. Also, for an infectious agent that has a wide host range, it may be necessary to reduce densities of an array of potential hosts. This can greatly increase the needed effort, particularly, when the hosts vary in their physical and habitat use attributes. However, failing to consider all potential hosts in control operations can result in failure of the entire management strategy.

Understanding the timing of the potential influx of susceptible individuals into the host population is also a key design consideration. Population sizes can increase throughout the year as new individuals are born or immigrate into the population. These demographic processes can ameliorate the effects of density reduction efforts. Density reduction efforts must account for potential influxes of individuals as well as the timing of when reproduction and immigration are likely to occur. Populations that have low reproductive rates and do not experience extensive immigration from other populations are generally better candidates for density reduction (Wobeser 1994). Similarly, density reduction of migratory species needs to be carefully designed to ensure

the “right” individuals or population are the target of removal, and those that are merely transiting through a region are not needlessly removed. Additionally, the social structure of the host population can significantly influence how populations respond to density reductions. Some species can have highly structured social networks, and when individuals are removed, the perturbation to the social system can be quite dramatic. These perturbations can potentially hinder or even negatively affect disease control efforts. Thus, density reduction is likely to be most successful for populations that have limited social structure.

The habitat used by the species targeted for density reduction will also play a role in the success of efforts and needs to be incorporated into the design. Populations residing in high quality habitat not only will likely be larger, but also may be more quickly to recover from reductions. Therefore, populations in good habitat may require a greater reduction. Assessing the extent of suitable habitat also helps establish the required spatial extent of reduction efforts by defining the “population” of interest. For example, if there are multiple connected patches of suitable habitat across the landscape that act as a meta-population, reduction efforts may be required across all interconnected areas. Targeted surveillance will need to be conducted in each of these patches or subpopulations to understand the extent of disease and delineate where reduction should occur. The configuration of the suitable habitat may also guide where reduction efforts can be most effective. Depending on the characteristics of the landscape, it may be possible to create buffers of low-density areas around infected foci leveraging the juxtaposition of suitable and unsuitable habitat and targeting reduction efforts. In short, the habitat characteristics can provide both challenges and opportunities to density reduction and should be integrated into design discussions for disease control.

There are also several logistical considerations when instituting density reduction when the reduction is through lethal means. The first is how carcasses will be handled and disposed of during reduction efforts. It is imperative that biosafety protocols be in place to protect personnel or the public conducting the animal removal. This is to protect them from both the focal disease, but also any potentially unknown zoonotic pathogens the host may be harboring. Testing removed animals can help assess the success of the density reduction on disease processes. This requires the availability of an acceptable diagnostic test, and ensuring the proper samples are collected prior to disposal of carcasses. Disposal of carcasses (described previously) should also be planned well in advance of implementation.

A second logistical consideration is who will be conducting the density reduction. When density reduction is necessary for game species, hunters may act as valuable tools to achieve the requisite reduction in density. This can be extremely valuable as it does not require agency personnel, animals can be removed from private lands, and the hunter is responsible for proper disposition of the carcass. However, the effectiveness of hunting at achieving the desired level of reduction needs to be evaluated, and agency culling may be needed to supplement hunter efforts if they are not enough. Lastly, the length of time reduction efforts will be applied to a population must be specified at the onset of activities to gauge the level resource commitment and communicate goals of management to stakeholders. It is unlikely that a single density reduction effort will be sufficient to achieve the disease control objectives, and control efforts may need to occur for an extended period of time. Often it may be difficult to ascertain how long efforts will be needed prior to control activities, but mathematical models can help inform these estimates initially and later can be improved as knowledge of the system increases.

As described above for selective removal, density reduction can often be highly controversial. This is only magnified when the removal is not limited to those most at risk or the infected. Although for wildlife diseases that are zoonotic or may affect domestic livestock, it may be less

controversial, nevertheless whenever density reduction is planned a thorough assessment of the social implications and the development of a communication plan is critical. The need for engagement of scientists from a wide array of disciplines is also critical for density reduction efforts. We described a number of scientific fields that could be engaged for selective removal and will not duplicate their description but reiterate that reducing densities to control disease is a challenging endeavor given the ecological and epidemiological complexities of the wildlife systems. Working across disciplines is a key to achieving disease control.

There are numerous examples of the use of reduction of host population densities for disease control; however, it is much more difficult to find cases where the effects of the management actions were critically assessed. For example, hunting is widely used to manage deer populations in North America, and with the introduction of CWD into many regions it is also used for disease control. However, despite its wide-spread application there are few examples of this practice being rigorously assessed to determine its efficacy. Largely, this deficiency is due to lack of adequate data to conduct a rigorous assessment. This highlights the importance and need for determining the metrics of success before initiating disease control efforts. Additionally, measuring success is not only useful for ensuring resources are well-spent and maintaining public support for disease management, but it can also help guide refinements to management activities and identify when they are not having the desired effects. For example, to protect cattle populations from bovine tuberculosis, density reduction was conducted for European badgers (*Meles meles*), which are reservoir hosts for the pathogen. Unfortunately, efforts seemed to increase bovine tuberculosis prevalence in badger populations and in cattle herds surrounding the treatment area. The problem in the badger system centered around the incomplete understanding of the ecology of this host species. After intensive studies of badger populations, it is now believed that culling activities, which altered the sex/age structure of the population and consequently the likelihood and type of social interactions, resulted in social perturbation of this territorial species leading to more excursions outside normal social groups. The new social dynamics ultimately led to high disease incidence in subsequent years (Tuyttens et al. 2000, McDonald et al. 2008). The European badger system highlights the importance of understanding the ecological processes of host populations when attempting to manipulate host populations (Prentice et al. 2019). Another example where disease control has had deleterious effects on wildlife populations without improving the disease situation is management of rabies in vampire bats (*Desmodus rotundus*) in Latin America. Bats were captured and covered with an anti-coagulant paste. The bats then returned to the colony, and through self and social grooming the poison was administered to numerous individuals resulting in density reduction within the colonies. However, despite extensive and long-term treatment efforts, human and livestock cases of rabies continue, and exposure of bats to the rabies virus increased after culling (Streicker et al. 2012). It was surmised that the cause of the lack of positive effect of culling on rabies was that culling targeted adult individuals shifting the age-structure of colonies to younger more susceptible cohorts, and that transmission was potentially operating in a frequency-dependent manner (Streicker et al. 2012).

In contrast, density reduction was successful at eliminating foot and mouth disease from white-tailed deer (*Odocoileus virginianus*) in California. Agency personnel removed 20,698 deer over the course of a year, and the disease has not been detected since (Wobeser 1994). Culling has also been purported to have maintained low prevalence of chronic wasting disease in white-tailed deer in Illinois, USA. Localized culling by agency personnel has been conducted since 2003 in Illinois through targeted removal of deer from known CWD hotspots. The intent of the culling is to maintain lower deer densities in these areas. Comparing prevalence rates from Illinois to the adjacent state of Wisconsin where culling only occurred from 2003—2008, showed the maintenance of low prevalence rates in Illinois and in Wisconsin during the years of culling.

However, once culling ceased in Wisconsin prevalence rates increased and were significantly higher than those in Illinois (Manjerovic et al. 2014). It is important to note that the culling ceased in Wisconsin not because it was deemed ineffective, but rather because it was highly unpopular with the public and lost political support. Thus, it provides a good example of the impacts of the social dynamics on wildlife disease control.

Another interesting example occurred in Uganda. A large colony of Egyptian fruit bats (*Rousettus aegyptiacus*) inhabited the Kitaka Mine, an active mine in southern Uganda. In 2007—2008, four miners and two tourists at the mine contracted Marburg hemorrhagic fever, and unfortunately resulted in two fatalities. Epidemiological investigations found marburgviruses in the bat populations roosting in the mine. In response to these findings, but against recommendations of natural resource personnel, the miners actively exterminated the bats in the mine to reduce their risk of infection. Several years later the large marburgvirus outbreak in Ugandan history occurred in the nearby town of Ibana. Subsequent investigations once again implicated bats inhabiting the Kitaka mine as the source of human exposure. The bat population was only 1—5% of its original size; however, the active infection of the bats was nearly 3 times the infection rate of the population prior to extermination efforts. It was speculated that the initial depopulation resulted in the recolonization of the mine with susceptible individuals which led to higher infection rates and increased human risk (Amman et al. 2014). This example illustrates two key points about density reduction. First, acting without understanding the ecology of the system can be perilous with a high potential for unexpected consequences. Secondly, human perception of risk can be a powerful driver of how disease control is ultimately implemented, and, as in this case, fear can sometimes override scientific guidance to the detriment of both human and wildlife health. Thus, in some systems managing the human perceptions is as important as actually reducing density.

We conclude with an example demonstrating that harnessing natural processes can improve disease control activities. Specifically, this example shows how human efforts to control densities of host populations can be enhanced by predation on hosts. In Spain, wild boar (*Sus scrofa*) are a well-known reservoir host for bovine tuberculosis. Additionally, Asturias, an area in Northwestern Spain has a healthy wolf (*Canis lupus*) population, whereas in the southern regions of Spain wolves are absent. Using data on differences in prevalence of bovine tuberculosis in wild boar between these regions in combination with mathematical modeling, it was shown that areas with wolves had lower and more stable prevalence rates of bovine tuberculosis than regions lacking this predator species (Tanner et al. 2019). This example demonstrates how exploiting the interactions of species within a community can aid in disease control efforts and highlights how predator species can provide key ecosystem services that help protect livestock and achieve anthropogenic goals.

In conclusion, reducing densities is and will likely continue to be one of the most used tools for disease control in wildlife hosts. However, we argue that instead of using this practice as a default for all wildlife disease systems, it should be critically evaluated prior to the initiation of reduction activities to determine the likelihood of successfully achieving objectives based on an assessment of the characteristics of the agent, host and environment. It is also particularly important for this management technique to study and understand the social environment in which the reductions will occur. Lastly, there is a need to assess the efficacy of density reduction efforts to better characterize when this management tool is likely to be effective and when it is likely to fail, and all future disease control efforts should try to include metrics of success.

Treatment and Immunization of Host Populations

To this point we have focused on disease management actions that have been targeting demographic or epidemiological processes at the scale of the population. We will now describe control or preventative measures that target the individual in hopes of having population-level effects on disease processes.

Treatment

Pathogens in the host can be controlled through treatments (e.g., antibiotics and anthelmintics). Although treatment is commonly used for humans and domestic animals, difficulty in delivering treatments limits its usefulness for managing disease in wild animals. Treatment has been successfully used on some endangered species where population sizes are small and it is possible to capture, treat, and release animals. For example, red grouse (Lagopus lagopus) treated with anthelmintic to reduce infections with the nematode *Trichostrongylus tenuis* resulted in higher reproduction rates and increased numbers of young (Hudson et al. 1992). However, ongoing treatment is often necessary particularly if some infected animals do not exhibit clinical signs and serve as a reservoir for new infections or reinfection of treated individuals. Widespread continued use of chemicals can also exert selective pressure for resistant pathogens as has been seen for the *Plasmodium falciparum* the protozoan that causes malaria (Hyde 2007) and *Yersinia pestis* the bacterium that causes plague (Gailmand et al. 1997).

From a practical perspective, even if sick animals can be detected early on in the course of the disease there is often a paucity of effective delivery and the probability of success of treatment may be extremely low. The requisite handling and associated stress may outweigh any therapeutic benefits of treatment for wildlife. There also may not be a known effective treatment for the species in question. For example, few drugs are labeled for use in wildlife, and they may have different and unknown effects on different species. The risk of using drugs will vary on the chemical and what condition is being treated, and therefore this problem cannot be overlooked. Additionally, there may be a lack of adequate personnel or facilities for treatment, making it impractical. A final consideration is that exposure, infection and eventual recovery of sick individuals creates a class of immune individuals in the population, which can be important for epidemic burnout as well as protecting the population from subsequent exposure to the agent. Short-circuiting the process through treatment may eliminate this population protection, if it reduces the individual's immune response and the number of immune animals in the population.

Despite treatment being uncommon, situations where it may be a preferred disease control approach include (Wobeser 1994):

- Treatment can be efficiently done for a large proportion of the population
- When an individual is of particular significance such as when managing species of conservation concern
- Treatment is conducted prior to the capture and translocation of animals
- Adequate resources are available for treatment programs without drawing them from other critical wildlife management programs

- Treatment is used to train personnel or harness public concern and gain support for disease management

For example, the treatment of free-ranging animals may be wise after capture and prior to release into a new area. In this situation, the goal is to prevent the spread of agents into populations already inhabiting a region, or to create populations free of the agent in new, uninhabited locations. Such treatments are often followed by a quarantine period and subsequent release, if the animals are demonstrated to be free of the agent. The failure to consider pathogen movement during translocation can have far-reaching negative consequences as demonstrated by the epidemic of rabies in raccoons in eastern North America (Nettles et al. 1979), the massive extinctions of tropical and temperate amphibian species due to chytrid fungus (Cheng et al. 2011), and the precipitous decline of the European crayfish after introduction of a fungal pathogen on introduced American crayfish (Alderman 1996). The 3rd Cycle Training Manual on Wildlife Health Risk Assessment in Support of Decisions and Policies provides a detailed discussion of risk assessments and management actions for wildlife translocations, and is a good resource for evaluating treatment as a management option in these situations. One important point to remember is that treatment should never be relied on solely for preventing spread of an agent via translocation (Wobeser 1994). It may be impossible to know for certain if treatment has been 100% effective, and as mentioned previously the efficacy of drugs in wildlife is generally unknown.

The most valuable outcome of treating individuals may be its impacts on humans. The treatment of sick wildlife may be an important learning opportunity to understand the biology, ecology and impacts of disease on a species, as well as, in the development of effective treatments that could be applied at scale. This training may be otherwise unavailable to the detriment of having a well-educated wildlife disease workforce (Wobeser 1994). Similarly, rehabilitation or treatment of individual animals may allow an agency to harness public concern about an outbreak or health event to promote conservation and health of species. For example, the treatment of common species after an oil spill, may have no impact on the population. However, the return in public good will and education about the ecology of the system might have enough intrinsic value to justify such actions (Wobeser 1994). Lastly, treatment of a rare species may be the only option to protect them from extinction. The case of the white rhino (*Ceratotherium simum*) exemplifies this situation where extensive efforts have gone into the treatment and care of individuals, particularly, of the few remaining individuals of the northern subspecies.

In conclusion, although treatment is the standard for human and domestic animal care, for wildlife it is rarely feasible or likely to be impactful. However, there are situations where this management technique is worth the investment and may deliver real-world benefits.

Immunization

Immunization can be used to prevent infection or development of the disease and are a primary mechanism for controlling disease in modern human and domestic animal medicine. The underlying theory supporting immunization is the idea of reducing R0 below 1 to prevent an epidemic or to help it burn out by reducing the number of susceptible individuals in the population. This concept is called herd immunity and is generally the goal of most immunization programs. To achieve herd immunity, in simple compartmental models, typically the proportion of individuals that are immune either after recovering from infection or through immunization must exceed $1 - \frac{1}{R_0}$, which is known as the immunity threshold (Fine et al. 2011). Continuing to exploit our simple model example, we show the impacts of vaccination on population prevalence (Figure 7). In this case, immunization occurs in the susceptible compartment at a continuous rate after day 75 of an

outbreak. As seen in this figure, immunization can be an effective tool for wildlife disease control. Additionally, it has the benefit of not impacting the overall population size unlike many of the previous management tools discussed.

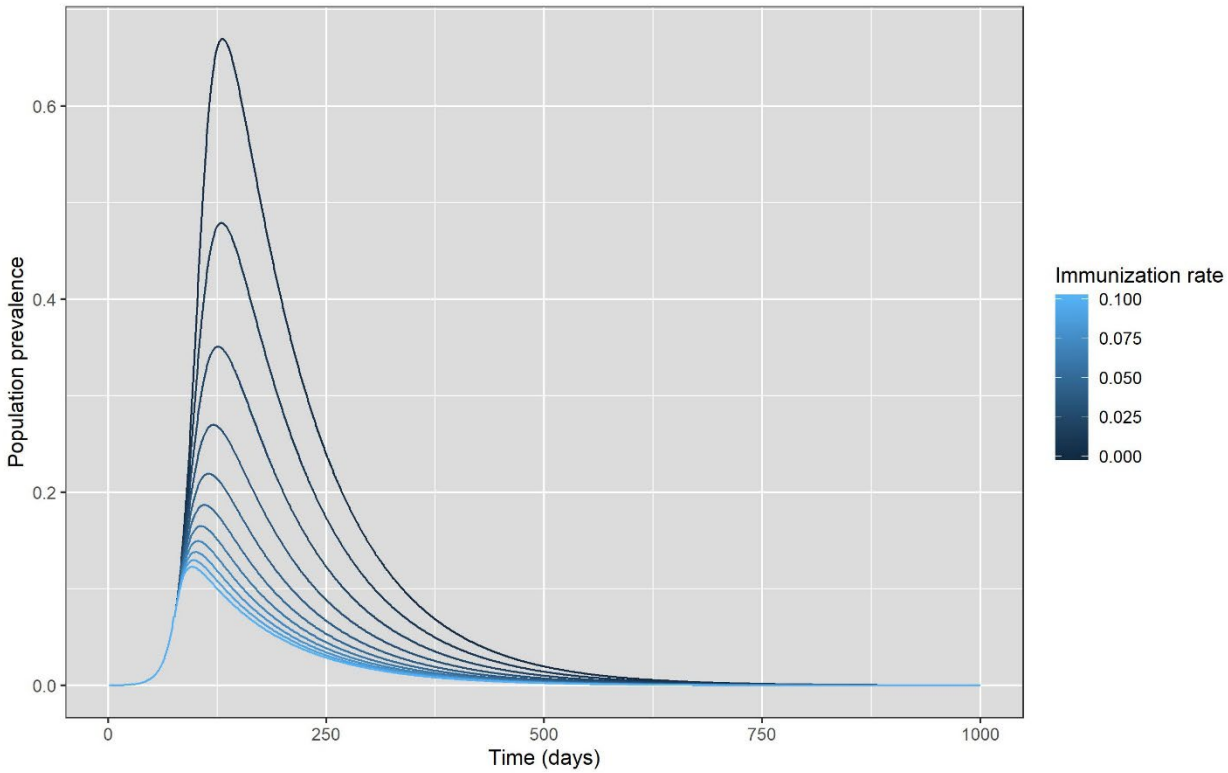


Figure 7-Impacts of a continuous immunization program on population prevalence.

Depending on the species and/or the delivery method, it may be only possible to immunize the population once. Figure 8 shows the impacts on population prevalence of a single vaccination event in the susceptible compartment for various levels of immunization effort administered on day 76.

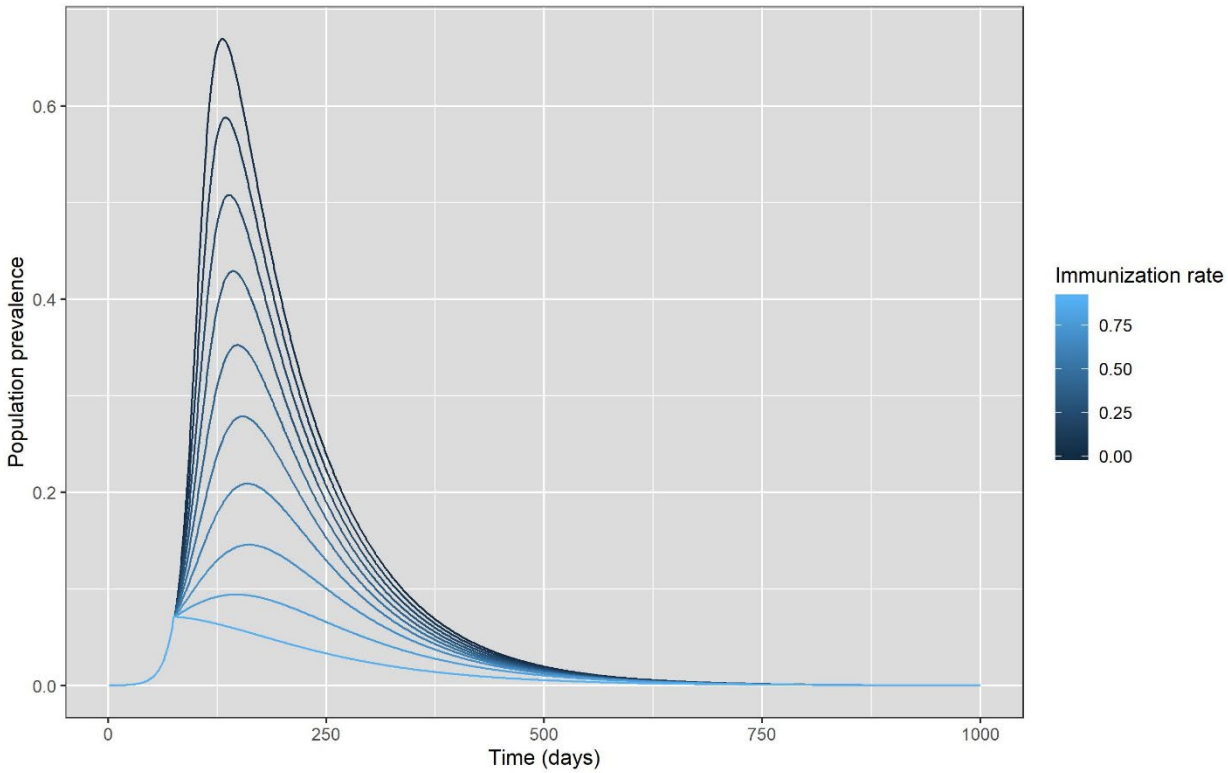


Figure 8-Impact of a one-time immunization on population prevalence.

Clearly, only vaccinating a population once has much less impact on population prevalence rate compared to a continuous program. A much larger proportion of the population must be vaccinated to have meaningful changes in the disease dynamics comparable to a continuous immunization operation.

Lastly, immunization efforts may be conducted prior to the arrival of disease (i.e., preventive vaccination). The impacts of this approach if applied one time before exposure are shown in Figure 9. Based on these results, preemptive immunization campaigns lower the maximum prevalence and flatten the prevalence curve.

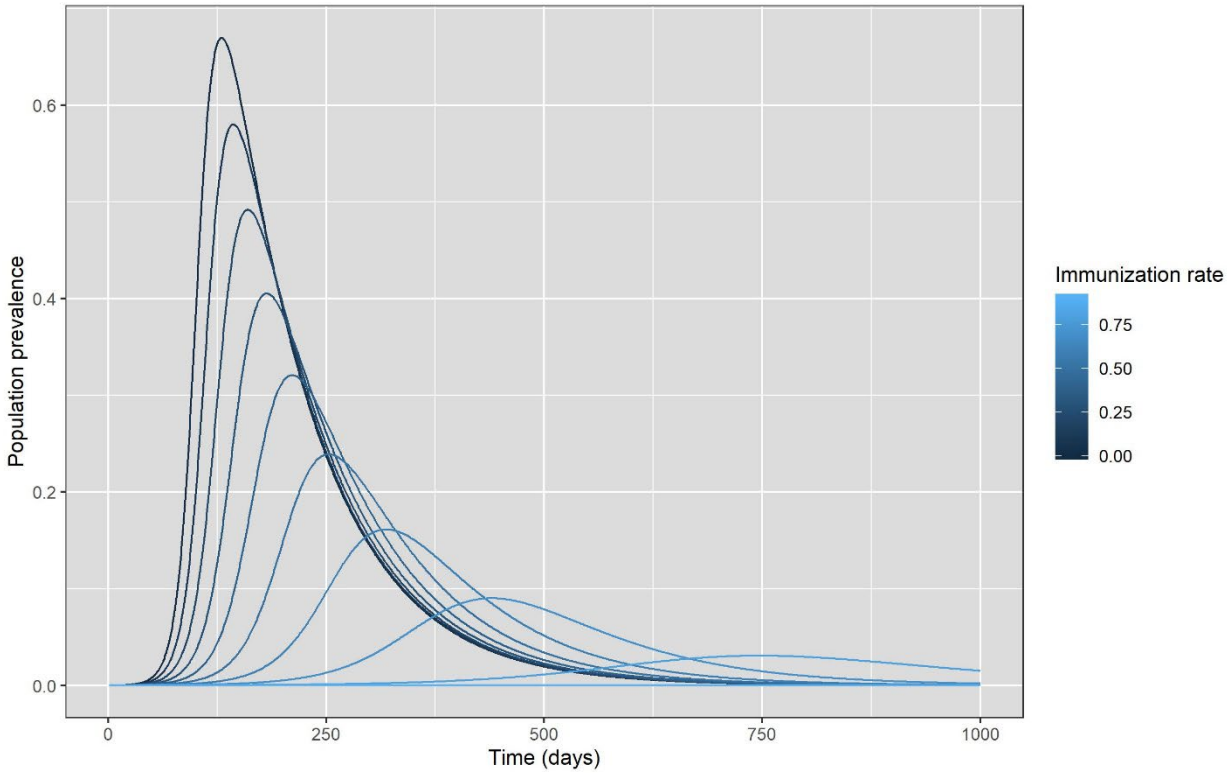


Figure 9-Impact of one-time vaccination prior to exposure on population prevalence.

As with all disease management options, immunization strategies have some basic considerations that need to be addressed to determine if they are appropriate and to properly design immunization efforts (Wobeser 1994). The first is the availability of a safe vaccine appropriate for use with wildlife species. An appropriate vaccine should stimulate a sufficient immune response in the host to provide protection from infection or disease and should not cause disease within the individual. Additionally, it is ideal if the vaccine is effective in a wide array of host species. Vaccines that provide protection from infection is preferred over protection from disease. In the latter case, animals may still contribute to infection of conspecifics and therefore the vaccine may be beneficial to the individual, but not the population. Thus, vaccines that prevent infection are more likely to change the course of an epidemic as shown above using our simple compartment model. Although if individuals are of high value (e.g., species of conservation concern), vaccines that prevent disease may still be useful. Also, vaccines that confer life-long protection against all serotypes or varieties of the agent are likely to be the most successful in managing the disease. If life-long protection is not conferred, individuals are able to become susceptible to the agent again, and therefore agents can potentially reinvade the population. It is also desirable that vaccinated animals be discernible from infected individuals. This is important for monitoring the intensity of disease, calculating the vaccination rate, and forecasting the impacts of control efforts. It is also critical if vaccination is being used with selective removal to ensure the right cohort of individuals is being targeted for removal. The vaccine also has to be stable and maintain its immunogenic potency under field conditions where it will be administered. Lastly, the vaccine needs to be safe for non-target species that might be exposed to it directly or indirectly.

Another important consideration is whether the vaccine can be delivered in an efficient way. Vaccines that require direct administration and require boostering are likely to be less useful for wildlife compared to those that can be passively delivered in a single dose. For example, vaccines that can be delivered orally, potentially through impregnated baits, will generally be more useful for application at a population scale than those where the vaccine requires direct handling or injection, particularly if handling requires multiple handling events (i.e., booster shots). Handling wildlife can be perilous to both the animal and the handler, and unintended injuries or mortalities may result. It is also important that if vaccines are delivered orally, such as through baits, the bait be of appropriate size that it is consumed by the host immediately in its entirety by the host to assure the individual receives the appropriate dosage. It will also help to deter individual hoarding of baits. Vaccines that can autonomously transfer among within the population are particularly useful for wildlife because treatment of a small proportion of the population is required to achieve herd immunity. This technology is currently being examined for use in preventing white nose syndrome and rabies in bats (Bakker et al. 2020). Lastly, if the vaccine does not confer perfect immunity or if immunity wanes this needs to be taken into account when determining what proportion of the population must be vaccinated. In fact, the immunity threshold needs to be inflated by $\frac{1}{E}$, where E is the effectiveness of the vaccine (Fine et al. 2011).

Assessing the characteristics of the agent can also help elucidate whether immunization is a feasible management option. Diseases that are caused by viruses, bacteria or protozoa (i.e., microparasites) are more likely to be suitable for vaccine development and application compared to those originating due to helminth or arthropod infections (i.e., macroparasites). The reason is that the former group of pathogens generally induce long-lasting immunity after infection, whereas the latter tend to result in persistent and recurring infections with the level of immunity correlating with the parasite load (Wobeser 1994). Therefore, herd immunity will be much more difficult to achieve when dealing with macroparasites because individuals can become susceptible again after infection. Additionally, agents that evolve slowly will be more amenable to control via vaccination. Some pathogens, such as influenza viruses, evolve rapidly permitting them to slip by the immunological defenses of the host. Developing a vaccine for these agents can be quite difficult because it will likely require continuous refinement to be protective (e.g., human flu vaccine). The mode and heterogeneities of transmission can also have significant impacts on the effectiveness of immunization programs, the required spatial distribution of immunization efforts, and when and for how long efforts will need to be applied. This means that if mathematical models are used to guide immunization programs, they need to incorporate the vagaries of the mode(s) transmission to appropriately capture the disease dynamics and accurately measure the impacts of vaccination (Antonovics 2017). For example, pathogens that have a high rate of spread will require a larger proportion of the population to be vaccinated and immunization should be rapid to maximize effectiveness of control efforts. The host range of the agent is also of particular importance for vaccination. If the pathogen infects multiple hosts with varying life history characteristics, it may be challenging to have an effective immunization program because the vaccine must be effective and deliverable to an array of species. Additionally, immunizing some host populations but not others is not likely to be effective in the long-term because there will always be reservoir populations which can serve as a source of infection for all hosts.

As always, the attributes of the host population will also be important to consider when planning immunization programs. The mean age of infection in hosts will determine the time frame during which immunization can be administered to be effective. If infection occurs early in the life of the host, there may not be adequate time to immunize individuals before they are infected. The most extreme example of this case is when a disease is vertically transmitted from the mother to the young, but other pathogens that infect young shortly after the waning of maternal antibody are

also equally problematic to address via immunization. The size and contact structure of the host population can also determine the effectiveness of vaccination. For example, large host populations that are spatially structured (e.g., territorial species) will require careful consideration on the spatial extent of immunization efforts, and the level of vaccination that is needed at each local site. Likewise, the rate of addition of susceptible individuals into the population will determine what proportion of the population will need to be immunized. Species that have high reproductive rates or populations that experience high immigration rates will likely require more effort to reach the immunity threshold because of the significant influx of susceptible individuals (Wobeser 1994). Predation may also be important driver of disease dynamics and can impact the feasibility of vaccination. If predation is focused on susceptible individuals (e.g., young), it can actually reduce the level of vaccination that is required. However, if predation disproportionately is removing vaccinated animals it may hinder immunization goals. For example, in heavily hunted populations there may be selection for the harvest of older animals, which may be more likely to be vaccinated or immune from past infection.

There are some also unique logistical considerations when it comes to designing and implementing an immunization program. One such factor, is the uptake rate if a vaccine is delivered passively. For example, if oral baits are used for vaccination, the uptake rates by the focal host as well as non-target species needs to be assessed to determine the proper density of baits to place on the landscape to ensure the desired proportion of the population is immunized (Tripp et al. 2014). Also, the length of time the immunization program needs to operate is critical. It may be in some situations where there is a persistent risk of the exposure that the immunization efforts will be required into the foreseeable future. The requisite resources and personnel must be available to sustain long-term vaccination efforts. Milestones and metrics of success must also be clearly established, particularly, if there will be long-term investment in the program. As mentioned previously, this means that there needs to be some means of distinguishing vaccinated individuals from those that have recovered from the infection. How the immunization program will be applied on the landscape is also an important design issue. Often there may be difficulties in vaccinating across the landscape as desired because of inability to access regions due to inhospitable terrain, private lands, extensive urbanization, etc. These constraints need to be addressed during the design to ensure adequate spatial coverage of the vaccine, or at a minimum a delineation of areas that may serves as sources of infection due to inadequate immunization. There also needs to be an economical means to manufacturer the vaccine and associated components needed for delivery (e.g., bait) to permit the immunization program to be implemented at the required scale. This is not a trivial consideration because if the vaccine is specific to a wildlife host there may not exist a readily available source of material, which means then that the materials must be manufactured by the agency. This can be a difficult and resource-intensive endeavor. Lastly, there are likely, depending on the country, numerous administrative hurdles that will need to be cleared prior to the use of a wildlife vaccine in the field. This includes showing the safety of the vaccine for non-target species. It can often take years to have a vaccine to be approved for wide-scale use in the field!

Socially, immunization programs often gain greater acceptance compared to those previously mentioned. This is likely due to the public's familiarity and general acceptance of vaccination for promoting human and domestic animal health, and the lack of individual animal removal with this management option. However, they also require some unique considerations. For example, if baits are going to be distributed for oral vaccination, it is important to inform the public in the region to provide awareness and prevent removal or ingestion, particularly by children or pets. Also, it is critical to educate the public about the safety of the vaccine for the focal species, but as well as non-target species such as companion pets. The public may also be valuable in implementing vaccination. For example, hunters and hunting clubs may be able to distribute oral

baits or aid in animal capture efforts. Lastly, as with the other management options described, developing and executing a good communication plan that keeps the public and decision-makers aware of the goals and results of the program is essential, especially for long-term immunization programs.

There are a few examples of large-scale vaccination programs for wildlife disease control. One such program is the vaccination program for sylvatic plague in western United States. Sylvatic plague is caused by the bacterium *Yersinia pestis*, and it can have significant impacts on and cause local extirpations of colonies of prairie dogs (*Cynomys spp.*). Prairie dogs are a key stone species in the grassland ecosystems and are the primary prey of the black-footed ferret (*Mustela nigripes*), a federally listed endangered species. Plague threatens ferret population through both direct mortality as well as indirectly through significant reductions of their prey base. Recently, an oral vaccine was developed and tested across 29 sites in the western United States (Rocke et al. 2017). The vaccine is a recombinant raccoon poxvirus engineered to express two protective *Y. pestis* antigens, F1 and a truncated V protein (Rocke et al. 2014). The vaccine was delivered via an oral bait containing peanut butter as the attractant and 0.25% Rhodamine B, a biomarker that is visible in hair, whiskers and feces of animals within 24 h of consumption. The marker was used to ascertain if an individual had eaten the bait and received the vaccine. It is worth noting that prior to wide-scale application, the vaccine went through extensive safety and efficacy trials before finally receiving USDA for experimental field use. Using information from previous testing (Tripp et al. 2014), baits were distributed at the sites at a rate of 100/ha. At each site, there were paired treatment and control plots. Control plots only received placebo baits whereas the treatment sites were treated with baits that contained the vaccine. Individuals were trapped, and a mark-recapture design was used to assess survival. This experimental approach was done to assess the efficacy of the vaccine. The results of this immunization program demonstrated that indices of abundance were higher on sites that were vaccinated, and at sites where plague occurred during the study apparent survival was lower on sites that received the placebo baits (Rocke et al. 2017). These results indicated the efficacy of the vaccine to provide at least partial protection for prairie dogs against plague. They also provided the impetus to begin large-scale application of vaccination throughout black-footed ferret habitat in 2019 with the distribution of 1 million baits over approximately 8,000 ha. To facilitate this immunization program, a private company was engaged to produce large quantities of baits. Additionally, technological advancements were made to efficiently distribute the bait, which was previously done by hand. These included the development of dispensing devices that work with drones and all-terrain vehicles. This immunization program provides a good example of many of the considerations that we described and overcoming many of the challenges with developing wildlife immunization programs.

Another example of a successful wildlife vaccination program is the use of oral vaccination to control rabies in wildlife. Rabies is a wide-spread zoonotic disease caused by RNA viruses in the genus *Lyssavirus,* family *Rhabdoviridae*. This disease is distributed widely across most continents and is typically found in meso-carnivore species that can serve as a source of infection for domestic animals and humans. Rabies vaccination efforts have a long history extending back to the 1970's. The current vaccine that is used is an attenuated recombinant vaccinia virus vector vaccine, and there are two currently two oral vaccine baits commercially produced and recommended by the World Health Organization (Maki et al. 2017). We will discuss the RABORAL V-RG® vaccine, but this does not constitute an endorsement of this product. Rather it was the product where detailed information was readily available and is widely used (i.e., over 250 million baits have been distributed globally). Extensive testing was done to ensure the safety, stability and efficacy of this vaccine (Maki et al. 2017). The vaccine is delivered via oral baits, and the baits are composed of a fish meal and/or fish oil. Baits are distributed on the landscape aerially, via hand-baiting or at bait stations depending on the species and country involved. The bait

includes a tetracycline marker to allow for detection of individuals that have consumed baits. Large scale oral vaccination campaigns have been successful in control rabies across the globe. For example, it was successfully used in France, Belgium and Luxembourg to eliminate rabies in red fox (*Vulpes vulpes*). It was also used in Ukraine to combat fox rabies and in Israel to control rabies in red fox and golden jackals (*Canis aureus*). In North America, extensive, long-term oral vaccination programs have been conducted to successfully control and limit the spread of rabies in raccoons (*Procyon lotor*), skunks (*Meles mephitis*), coyotes (*Canis latrans*), red fox and gray fox (*Urocyon cinereoargenteus; Maki et al. 2017*). There is no doubt that vaccination programs for rabies in wildlife are successful. This success has required extensive effort and resources not only during vaccine development, but also during field deployment where optimization of uptake for various species has received extensive study. Thus, it is clear that immunization can be a valuable tool for managing wildlife diseases when the resources and societal will to do so are sustained, and the oral vaccination campaigns for rabies across the globe are probably still some of the best examples of controlling wildlife disease through immunization programs.

In conclusion, immunization programs can be powerful and effective tools for controlling disease in wildlife species. They are generally supported by the public, and do not require removal of individuals. However, their use in wildlife can be challenging because of the need to often develop custom vaccines that are safe and stable, the regulatory burden associated with them, the need to develop an efficient delivery method, the often-lengthy time it takes before they are available for field use, and the need to often maintain long-term commitments to their application in a population. Thus, immunization programs have great potential, but they are not always the correct solution to every wildlife disease problem.

Combining Tools

We have described a variety of tools that can be useful to manage wildlife disease. We have also tried to detail, albeit only some, of the important considerations associated with the application of each technique. It is often the case that these tools can be used in concert to more efficiently manage the wildlife disease in question. For example, selective culling and vaccination programs may be more effective when applied simultaneously than when each is used in isolation. Therefore, when attempting to manage a wildlife disease issue, it is valuable to not only evaluate the suite of potential actions, but also examine if any can be applied synergistically to achieve better results.

An excellent recent example of where multiple tools have been used for wildlife disease management is the management actions taken to control CWD in Norway. In 2016, CWD was detected in wild reindeer (*Rangifer tarandus*) and moose (*Alces alces*) in Norway. The infection in reindeer was particularly troubling because Norway manages the last remnant of the wild tundra reindeer in Europe (Benestad et al. 2016). In response, the managing agencies took an aggressive approach to tackling this issue. In an attempt to eradicate classical CWD in Norway, the competent authorities, using hunting and agency culling, removed the entire herd of wild reindeer in the affected region near Nordfjella, where the first CWD case was detected. This consisted of the removal of approximately 2,000 animals of which 19 tested positive for CWD. The intention is now to allow the area to remain fallow for a minimum of 5 years before reindeer are reintroduced. During this time, efforts will be aimed at minimizing the use of the affected area by populations of other ungulate species through culling programs. Additionally, nearly 660 salt licks, which were believed to pose a risk of prion transmission, were fenced to prevent their use by wild ungulates while still allowing access to domestic sheep. The government also constructed > 24km of fencing to restrict access of wild and semi-domestic ungulates to the affected region.

Lastly, the government is maintaining an extensive targeted surveillance program for CWD throughout the country to monitor for the disease and measure success of control efforts (VKM 2017).

The above example demonstrates the use of multiple tools and methods to try and eradicate a wildlife disease. Culling, hunting, density reduction, fencing and extensive communication efforts have all been important components of the CWD response efforts in Norway. Although it remains to be seen whether this aggressive management style will succeed in disease eradication, there is much be learned from the successes and failures of this effort.

Table 4. Example assessment of the application of two management tools for manipulating host distribution

ASSESSMENT OF MANAGEMENT ACTION				
KEY				
👍	management action is suited			
👈	management action unaffected or equivocal			
👎	represents challenge for management action			
			Distribution Alteration	
Compartment	**Characteristics**	**Result**	**Dispersal**	**Fencing**
Agent	Endemic	Yes	👈	👍
		No	👍	👈
	Novel to the system	Yes	👍	👈
		No	👈	👎
	Localized	Yes	👍	👍
		No	👎	👎
	Emergence mediated by environment	Yes	👍	👍
		No	👎	👎
	Vector-transmitted	Yes	👈	👎
		No	👍	👍
	Directly transmitted	Yes	👎	👎
		No	👍	👍
	Indirectly transmitted	Yes	👍	👍
		No	👈	👎
	Human-assisted transmission/spread	Yes	👎	👎
		No	👍	👍
	Affects multiple hosts	Yes	👎	👎
		No	👍	👍
	Rate of transmission	High	👎	👎
		Low	👍	👍
	Seasonal effects	Yes	👍	👈
		No	👎	👈
Host	Large population	Yes	👎	👎
		No	👍	👍
	Migratory	Yes	👍	👎
		No	👈	👍
	Mobile	Yes	👍	👎
		No	👎	👍
	Easily hazed	Yes	👍	👈
		No	👎	👈
	Interspecific interactions	Yes	👎	👍
		No	👍	👈
	Herd animal or lives in large groups	Yes	👎	👈
		No	👍	👈
	Behavioral challenges for fences	Yes	👈	👎
		No	👈	👍
	Small in size	Yes	👈	👎
		No	👈	👍
	Potential interactions with domestic animals	Yes	👎	👍
		No	👍	👈
	Complex social structure	Yes	👎	👎
		No	👍	👍
	Conservation concern	Yes	👍	👍
		No	👈	👈

Table 4. Continued

Environment	Management will impact non-target species	Yes	👎	👎
		No	👍	👍
	Habitat alteration necessary	Yes	👎	👎
		No	👍	👍
	Available habitat outside affected area	Yes	👍	👍
		No	👎	👎
	Environment serves as point source or reservoir	Yes	👍	👍
		No	👎	👌
	Accessible for management	Yes	👍	👍
		No	👎	👎
	Long-term impacts on structure	Yes	👎	👎
		No	👍	👍
Logistics	Resources available for long-term application/maintenance	Yes	👌	👍
		No	👌	👎
	Human infrastructure in the reigion	Yes	👌	👎
		No	👍	👍
	Needs to be implemented rapidly	Yes	👍	👎
		No	👎	👍
	Other control tools available	Yes	👌	👌
		No	👍	👍
	Necessary expertise available	Yes	👍	👍
		No	👎	👎
Social	Public is aware of management actions	Yes	👍	👍
		No	👎	👎
	Public is resistant to management	Yes	👎	👎
		No	👍	👍
	Communications plans can be developed	Yes	👍	👍
		No	👎	👎
	Political support exists	Yes	👍	👍
		No	👎	👎
	Livestock interests in management	Yes	👎	👍
		No	👍	👍
	Human health concerns	Yes	👎	👌
		No	👍	👍
	Special interest groups that need to be engaged	Yes	👌	👌
		No	👍	👍

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