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Surveillance Strategies For Detecting Chronic Wasting Disease In Free-Ranging Deer and Elk[1]

Results of a CWD Surveillance Workshop

Madison, Wisconsin December 10-12, 2002

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[1] This document is available on the NWHC website: http://www.nwhc.usgs.gov/research/chronic_wasting/CWD_Surveillance_Strategies.pdf

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Executive Summary

Chronic Wasting Disease (CWD), a fatal brain disease of North American deer and elk, has recently emerged as an important wildlife management issue. Interest and concern over the spread of this disease and its potential impact on free-ranging cervid populations has increased with discovery of the disease in numerous states and provinces. Current studies suggest that CWD may adversely affect of these highly visible, socially desirable, and economically valuable species. Despite the lack of evidence that CWD affects humans or livestock, a significant concern has been the perceived risk to humans and livestock. Uncertainty about whether CWD poses a health risk to hunters and their families who consume venison has resulted in testing of free-ranging cervids for CWD. In response to many of these concerns, wildlife management agencies across the nation have undertaken surveillance programs to detect CWD in their cervid populations. The nation-wide costs for an extensive CWD surveillance program have been estimated at several million dollars.

This document provides guidance on the development and conduct of scientifically sound surveillance programs to detect CWD in free-ranging deer and elk populations. These guidelines will not apply equally to all jurisdictions. In many cases local circumstances, resources, area(s) of concern, disease risk, animal and landscape ecology, political, social, and many other factors will influence the objectives, design, and conduct of CWD surveillance programs. Part I of this report discusses the importance of management goals, strategies, and disease risks in developing a surveillance program. Part II describes surveillance methods, steps in designing a sampling strategy to detect CWD, alternative collection methods, and statistical considerations. Part III describes costs (personnel, time, and money) associated with implementation of these plans that will influence program design. Part IV outlines research that is needed to further development of CWD surveillance methods. Unfortunately in dealing with CWD, many important biological facts are still unknown and further research will be required to answer these questions. In most situations surveillance strategies suggested may require several years to complete, will require careful consideration of management objectives, and extensive operational planning in order to be meaningful and to be scientifically based.

Chronic Wasting Disease Surveillance Workshop

The US Geological Survey's National Wildlife Health Center convened an interdisciplinary, inter-agency group for a 3-day workshop in Madison, Wisconsin to develop guidance for surveillance strategies for CWD in free-ranging deer and elk. Participants represented a cross section of scientific expertise in statistical sampling; cervid ecology; epidemiological, management, and operational aspects of CWD; wildlife disease surveillance programs; and in the types of settings (federal lands, states, tribal lands) in which surveillance is likely to be conducted. The mission of the workshop was to provide a technique-oriented focus for designing, developing, and implementing CWD surveillance programs for free-ranging cervids.

This workshop was organized to help address growing concerns and uncertainty about the increased recognition of CWD in free-ranging deer and elk throughout North America by developing guidance for agencies that wish to conduct surveillance programs for CWD within their jurisdiction. Goals of the workshop were to:

1) define surveillance goals for the management of CWD in free-ranging populations,
2) identify procedures and statistical methods to meet surveillance goals,
3) identify key operational components for collection of animal samples from a surveillance program, and
4) identify research needed to improve surveillance programs.

The workshop was organized into a half-day series of key presentations related to CWD management goals, operational components of a surveillance program, cervid ecology, disease modeling, and statistical sampling (see meeting objectives in Appendix 1). The meeting included a half-day tour of ongoing Wisconsin Department of Natural Resources CWD deer collection and processing operations. The remainder of the workshop was spent in moderated discussions that focused on developing guidance and recommendations for CWD surveillance programs.

Participants

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[3] Workshop facilitator

Background and Assumptions

In developing surveillance programs for CWD in free-ranging cervids it is important to consider several key factors. First, our scientific understanding of the ecology and transmission of CWD in free-ranging wildlife is very limited. Although this report reflects current knowledge, we assume that ongoing and new research will improve the scientific basis for understanding and managing this relatively new disease. As new information about the epidemiology of CWD is obtained many aspects of this report should be carefully reconsidered. Second, surveillance activities must be closely integrated with management actions and scientific investigations. A scientifically sound surveillance program is critical to providing data for making management decisions, and can play a key role in helping to better understand the ecology of CWD in free-ranging populations.

This report covers the series of decisions and programs that need to be considered when developing CWD surveillance plans for free-ranging animals. It begins by considering the management goals and responses that will be needed if the surveillance program finds CWD. These components should be considered before implementing a surveillance program. In conjunction with the management goals, objectives of the surveillance program need to be carefully developed and evaluated. Operational aspects and costs of the surveillance will play an important role in determining sampling design and animal collection and testing methods.

CWD surveillance programs may include three objectives:

1) detection of disease in areas not known to be affected,
2) assessment of the spatial distribution and prevalence in CWD affected areas, and
3) monitoring changes in CWD over time, in response to management actions or in conjunction with research programs.

These objectives represent the typical progression that might occur when moving from absence of disease, to discovery of disease foci, to ongoing management of CWD in populations. Although there are many common steps in developing surveillance programs to meet these different objectives, many aspects of the surveillance design, conduct, and interpretation will be unique to each situation.

Workshop participants concluded that it would not be feasible to fully consider all three objectives for a surveillance program. As a result, this report does not cover surveillance activities to assess the distribution, extent, or prevalence of CWD in affected areas, nor does it cover surveillance programs to monitor changes in these factors in areas where CWD has become established. This report is primarily concerned with surveillance programs to detect CWD in areas where it is not known to occur. However, many components of the process described in this report will be applicable to developing surveillance programs that focus on assessment or monitoring of CWD.

We made a number of initial assumptions in structuring our deliberations. These assumptions are subject to revision pending ongoing and future research on the epidemiology of CWD in cervids. These assumptions include:

- ***Approach to surveillance***: CWD surveillance occurs under widely varying conditions related to animal and landscape ecology, animal densities, political and cultural factors, and fiscal and personnel resource considerations. No prescriptive formulas will apply to every circumstance. There are, however, essential steps to be considered and clearly documented to help determine the most appropriate surveillance strategy for a particular set of circumstances.
- ***The disease***: Our understanding of the epidemiology of CWD is incomplete; definitive information on transmission, initial causation, and other important factors is currently unavailable. CWD is a prion disease related to other transmissible spongiform encephalopathies (TSEs). It is currently known to affect North American species of mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), and elk (*Cervus elaphus*). It is transmissible by contact with the agent, through direct (animal-animal) contact, and indirectly through the environment (animal-environment-animal). The disease has a long incubation period (> 15 months) and progression varies by species. Clinical signs only appear in the final months before death. The agent is primarily spread to other areas by the movement of live animals, but other mechanisms may also contribute. On a local scale (e.g., county or game management unit) the disease occurs at low prevalence; however, within the affected area, clusters of infected animals with much higher prevalence rates are typical. The disease spreads slowly through wildlife populations compared with other infectious diseases, yet outbreaks can be self-sustaining and prevalence tends to increase over time. Based upon current patterns, however, risk factors can be identified. Review papers on CWD have been published[4,5]. Additional information including basic questions and answers and related bibliography can be found at a variety of websites[6,7].
- ***Disease testing:*** For the foreseeable future, the immunologically-based immunohistochemical (IHC) stain is the most reliable test for the detection of CWD in animal tissues. Testing is a keystone issue in any surveillance program and it must be reliable to provide an accurate basis for future activity. Alternative screening tests have been developed and approved that may reduce the costs and/or increase the rate at which tests can be conducted. Always consider a confirmatory test, such as the IHC, for CWD-positive tissues, especially when confirming disease in a new area.

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[4] Williams, E. S., J. K. Kirkwood, and M. W. Miller. 2002. Transmissible spongiform encephalopathies. Pp. 292-301 in E. S. Williams and I. K. Barker, eds. Infectious Diseases of Wild Mammals. Iowa State University Press, Ames.

[5] Williams, E. S., M. W. Miller, T. J. Kreeger, R. H. Kahn, and E. T. Thorne. 2002. Chronic wasting disease of deer and elk: a review with recommendations for management. Journal of Wildlife Management 66: 551-563.

[6] http://www.nwhc.usgs.gov/

[7] http://www.aphis.usda.gov/vs/nahps/cwd/

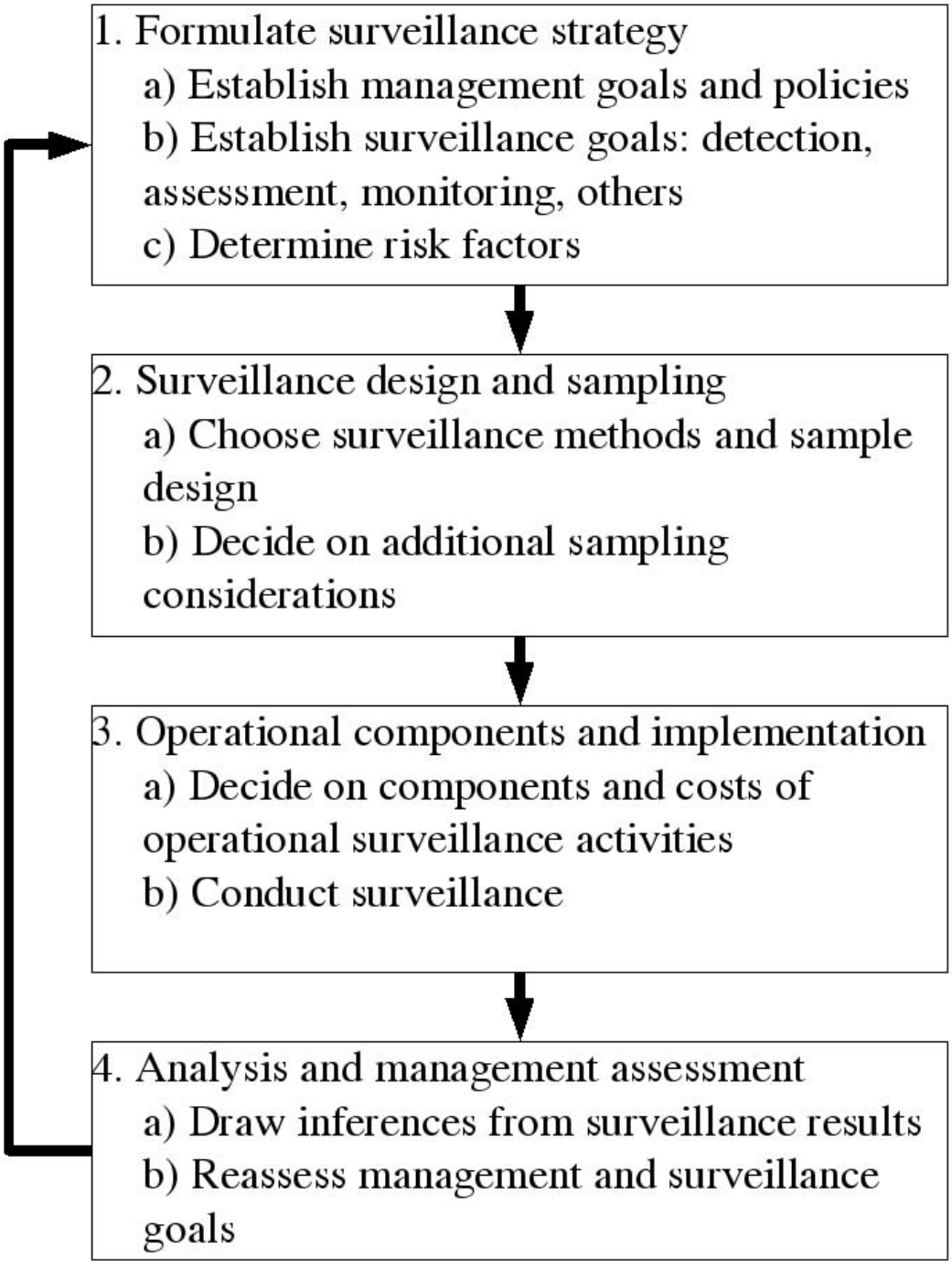
- ***Integrated surveillance*:** The focus of these guidelines is on surveillance for free-ranging deer and elk, but CWD also occurs in farmed or captive herds. In many circumstances, surveillance strategies and results for both free-ranging and farmed/captive herds need to be closely integrated. Elk and deer and the diseases that affect them show little respect for differences in ownership or jurisdictional boundaries[8] and disease may spread between captive and wild populations.

This report is organized into four parts. Part I covers development of surveillance goals and evaluation of disease risk factors, and outlines steps that should be considered and documented when establishing management goals and objectives of a surveillance program. Part II outlines the recommended steps in designing a sampling strategy to detect the presence of CWD and alternative statistical, sampling, and collection strategies that should be considered to meet the surveillance goals. Part III describes the components and estimated costs of an operational surveillance program. Part IV outlines research needs for further development of CWD surveillance methods. A glossary at the end of the document defines many of the terms used herein.

Rather than a standard approach to CWD surveillance, this report emphasizes the importance of linking surveillance goals with management; development of a sound surveillance program that will provide scientifically based results that meet program objectives; and careful execution of the surveillance plan. Diagram One identifies a series of four steps that can be followed in developing a surveillance strategy for most circumstances. Depending on the results obtained from the surveillance program, it may be necessary to reconsider all aspects of the surveillance program. In essence, CWD surveillance should be considered a dynamic rather than a static process. The following sections provide general principles, examples, and references for carrying out each step.

[8] In this report, the term "jurisdiction" refers to States, Federal land management units, Tribal lands and their appropriate authorities.

Diagram One: Steps in Conducting CWD Surveillance



I. Formulating a CWD Surveillance Strategy

In designing surveillance strategies and methods you should consider how to integrate CWD surveillance within an overall program that includes goals and policies for managing CWD. In developing a CWD surveillance strategy, it is important to think beyond the surveillance program to the likely management actions that will be instituted and the potential programmatic impacts that will occur if CWD is detected. Considering these factors will help focus the scope, time frame, and extent of surveillance needed to address these management concerns. Prior development of a CWD contingency plan may be a useful tool to guide this process. In addition, because of public concern about CWD, surveillance design and methods (as well as other CWD policy and management components) must reflect a transparent decision making process.

Establishing Management and Surveillance Goals

Management and surveillance goals for CWD are separate but closely related issues. A number of management plans have been developed by states and other agencies to deal with the presence or potential presence of CWD in wild deer and elk populations[9]. In general the management goals include: 1) prevention of CWD (e.g., through reduction of risk factors), 2) control or containment of CWD (e.g., through reduction of herd size), 3) elimination of CWD (e.g., through eradication of herds), and 4) monitoring for prevalence, distribution, and mortality of CWD in a population[10,11]. A variety of management goals exist because CWD management occurs within the jurisdiction of the relevant state, federal, and tribal management agencies. In addition, management goals and plans may be influenced by the extent and intensity of disease, as well as economic, social, and political factors. Because CWD is a relatively new disease in most areas and because our scientific knowledge is generally limited, the best management programs to achieve these goals have not been determined.

When possible coordinated CWD management should encourage cooperation among jurisdictions that border or overlap each other. Furthermore, although this report focuses on surveillance for free-ranging deer and elk, CWD also occurs in farmed and captive herds. In many circumstances, surveillance strategies and results for both free-ranging and farmed/captive population*s* should be closely integrated.

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[9] For example: http://gf.state.wy.us/HTML/hunting/CWDplan.htm, http://wildlife.state.co.us/CWD/index.asp, Nebraska Game and Parks Commission. 2002. Chronic Wasting Disease Management in Nebraska. Wildlife Division, NGPC. Lincoln, Ne. 8 pp.

[10] A full discussion of CWD and management strategies is contained in Williams, Elizabeth, et.al., 2002, Chronic Wasting Disease of Deer and Elk: A Review with Recommendations for Management. Journal of Wildlife Management 66(3): 551-563.

[11] Plan for Assisting States, Federal Agencies, and Tribes in Managing Chronic Wasting Disease in Wild and Captive Cervids. U. S. Department of the Interior and U. S. Department of Agriculture. June 26, 2002.

Table One describes three goals for CWD surveillance: detection, assessment, and monitoring. These surveillance objectives likely will differ depending on the management goals (prevention, elimination, monitoring, and control) and whether or not CWD has been found.

Table One: Management Goals and Surveillance Objectives

Management Goals	Prevention or Elimination	Elimination, Monitoring, or Control	
Surveillance Goals	Detection	Assessment	Monitor
Surveillance Objectives	Establish whether CWD occurs in a jurisdiction or part of a jurisdiction; If not detected, estimate likelihood that CWD is absent	Determine the spatial distribution and prevalence of CWD in the target population	Estimate change in prevalence, rate and direction of spread/contraction; Research to understand epidemiology (how CWD is transmitted through a particular target populations); Measure and evaluate the effect of management actions

***Detection*:** Surveillance strategies to detect CWD should consider the potential management actions that will follow. Surveillance programs that can detect CWD early, when the disease is present in only a limited number of animals, will provide the best opportunity to eliminate the disease. The detection phase determines the CWD status of a free-ranging target population or a geographic area. Either CWD was found during the surveillance, and thus is present or CWD was not detected given an assumed prevalence. In the event CWD is not found, it is important to estimate the likelihood that CWD prevalence is less than a specified level. In many areas and/or target populations, surveillance will not detect CWD. Without complete depopulation and testing we can't demonstrate with 100% certainty that CWD is not present in the target population; however, well designed sampling can achieve a high degree of confidence that disease is not present above a selected prevalence. Claims of being "CWD free" should be avoided unless all animals in a target population have been tested. Statements about the confidence levels for detecting CWD should be based on surveillance programs that consider the probability of detecting affected animals, the test methods used, methods for collecting representative animals, surveillance and sample designs, the prevalence of disease that could be detected in the population/area, and the number of animals

represented by this prevalence rate. Given our current knowledge, claims of "CWD absence" should be carefully evaluated. This important topic is discussed later in this report.

If management goals are primarily to monitor disease occurrence, changes in prevalence, and impacts of the disease on deer and elk populations, then less intensive surveillance programs may be acceptable.

***Assessment*:** If CWD is found, management agencies may consider using an assessment strategy, especially if elimination or control is the management goal. Assessment determines the geographic extent of disease and distribution of disease prevalence so that appropriate management responses can be determined. In assessment, a much more intensive and systematic surveillance strategy may be needed in the region (or target population) where the disease is expected to occur. Intensive surveillance strategies can be designed to obtain the required number of samples in a relative short period of time; less intensive efforts can allow sampling over a longer period of time. Following a CWD-positive diagnosis, political and public pressures for a management "solution" may complicate the surveillance program because management actions may take precedence over a more thorough assessment of the disease situation.

***Monitoring*:** After an assessment establishes a baseline of disease occurrence or distribution, surveillance goals could shift to monitor the situation and address one of more of the following questions:

- "Is there change in the prevalence of disease?"
- "Is there change in the rate of disease transmission?"
- "Is there a change in the spread or contraction of CWD over the landscape?"
- "How is CWD being transmitted and spread in the population?"
- "What has been the impact of the management actions taken?"

Monitoring for changes in disease patterns can be particularly valuable when linked with research to understand the epidemiology of CWD. In these situations monitoring programs must be closely linked with the objectives of the research program being conducted. Monitoring is also an important component of agency programs that are being conducted to manage CWD. Monitoring changes in disease patterns and impacts of disease on target populations provides the primary source of information to assess the effect of management programs and is a crucial component of monitoring target population response to adaptive management approaches for CWD.

As one moves from surveillance for detection of disease to assessment to monitoring, the complexity of the surveillance strategy, methodology, and analyses generally increases. Although some components of a surveillance program are common to each of the surveillance objectives, here we will limit discussion to surveillance for detection of CWD.

Determining Risk Factors

Risk factors are attributes of the landscape, environment, or animals associated with a greater probability of CWD occurring in a target region or target population. Establishing the presence (or absence) of risk factors is fundamental for focusing attention and allocating resources in any large-scale surveillance strategy. This is particularly important for CWD because in most areas disease is likely to occur at a low prevalence that is difficult to detect and the disease is not evenly distributed over the landscape. Current information suggests that CWD occurrence and prevalence can vary among geographic areas (states), among regions within states, and occurs in disease clusters of affected animals within these regions. As a result, surveillance to detect CWD without reference to potential risk factors is likely to be inefficient (Appendix 3). At the current time, our knowledge of the risk factors is limited; a better understanding of risk factors is needed to improve the efficiency of surveillance programs.

Table Two lists major CWD risk factors in two groups; related to exposure (introduction of the disease into a new area or target population) and related to amplification (spread of disease through a target population or a region). As stated previously, the CWD agent is thought to be transmitted by direct animal contact or indirectly through its presence in the environment. The risk of free-ranging animals being exposed to CWD is, therefore, greater in areas where CWD-positive animals have already been found. Further, movement of infectious animals or materials across the landscape, naturally or with human assistance, increases the exposure risk to uninfected populations. The frequent movement of farmed elk and deer between production facilities, the animals' concentrated presence on such facilities, and the possibility of their escape into the wild increases the risk of spreading CWD to uninfected populations of free-ranging animals. Because the infectious agent likely persists in the environment, the introduction of noninfected animals (either captive of free-ranging) into a contaminated environment could increase the risk of infection. Even locations from which CWD-positive animals have been removed may remain contaminated.

Once exposure occurs, the risk of amplifying the disease (increasing the number of infected animals) in a target population or location likely increases with higher elk or deer population density as well as habitat and other ecological characteristics that influence animal distribution, movements, and behavior. The absence of predators may allow sick animals a longer period in which to spread CWD. Baiting or feeding increases concentrations of animals and may increase the chance of disease spread through direct contact among animals or indirect contact with environmental contamination. Contaminated environments may serve as a source of infection to animals for extended periods.

Table Two: Known or Suspected CWD Risk Factors

Exposure Risk Factors	Areas adjacent to CWD-positive wildlife
	Areas adjacent to land on which TSE-positive animals, farmed or wild, have lived
	Areas with CWD-positive farmed or captive herds
	Areas with concentrations of farmed or captive elk or deer
	Areas that have received translocated deer or elk from CWD-affected regions
	Areas permitting transport of hunter-killed elk or deer carcasses from CWD infected areas
Amplification Risk Factors	Areas with high elk or deer population density
	Areas with a history of CWD animals or CWD contaminated environments
	Areas with low abundance of large predators
	Areas where free-ranging elk or deer are artificially concentrated (baiting, feeding, water development, and other human related habitat modifications)

Evaluation of risk factors helps to focus resources on locations or target populations with a greater likelihood of being infected and increases the efficiency of surveillance efforts. Presently, our ability to quantify the importance of risk factors is limited and determination of their importance for any specific area must rely on the judgment and experience of experts. Surveillance on and around CWD-positive elk or deer farms or farms that have received animals from known CWD areas, and along the borders with other jurisdictions with CWD-positive animals can increase the effectiveness of surveillance efforts. Additional risk factors, such as the presence of scrapie in sheep populations that are sympatric with deer and elk, feeding of animal protein to elk or deer, baiting and feeding programs, or environmental factors also may be considered although their role in CWD epidemiology has not been clearly established. Understanding the distribution, movement, social behavior, population characteristics, and dynamics of affected deer and elk populations is helpful, if not essential, to fully evaluate the risk factors for CWD in free-ranging populations.

II. Surveillance Methods and Sample Design for CWD Detection

A variety of surveillance methods and sample designs are available for CWD surveillance. Each has positive and negative aspects; the program you design should meet the goals, risks, and resources for your situation. Your options will depend on management and surveillance goals, risk factors, and resources required. It may be useful to look at surveillance methods and sample design as a set of discrete decisions that should be interconnected with the goals, risks, and resources for each surveillance program. These decisions are not generally made sequentially but rather interact with

one another throughout the process. Thus cost and resources, discussed later in this section, may be a major parameter in determining extent and type of surveillance strategy or type and number of animals collected for CWD testing. The challenge is to decide which strategy will make the best use of that resource, given a specific surveillance goal and risk.

This report emphasizes three steps in developing a CWD detection surveillance program:
1) defining the target population and geographic region,
2) identifying and selecting sampling units, and
3) determining sample size and methods of collecting disease data from the sampling units.

Each of these steps is discussed below. Appendix 2 features an expanded discussion of relevant surveillance methods and sampling designs.

Region and Target Population(s)

The most practical approach to surveillance of free-ranging wildlife is to define a region(s) to sample and identify the target population(s) contained in that region. The region will often be a political jurisdiction: a state, a tribal land, a national park or refuge. The size, landscape, environmental conditions, number and distribution of animals in the target population(s), animal ecology and movement patterns, and location of this region are important variables to consider when developing a surveillance strategy. If distinct target populations can be identified, it is usually preferable to define sampling regions ecologically to encompass complete target populations, thus allowing conclusions about the region to be applied to the target populations.

In some regions, surveillance may need to consider separate target populations of animals. In the western US, several distinct populations of deer and elk, associated with different winter ranges or complex of habitats, may occur within a single geographic region. These populations may have different migration patterns, behaviors, population demographics, management regulations, and disease risk factors.

Though elk, mule deer, and white-tailed deer may occupy the same general area, data on CWD are best tracked separately for each species or target population, rather than considering all cervids as one target population. Existing information demonstrates that rates of infection vary among cervid species, possibly due to genetic susceptibility, different rates of disease transmission, and/or differing social behaviors. However, transmission of CWD is likely to occur among sympatric cervid populations.

Finally, it is crucial to consider the size of the region and number of animals in relationship to the surveillance objectives for detecting CWD. To assess this we believe it is important to calculate the number of CWD infected animals that could be detected within the target region and evaluating this in the context of the management goals (prevention, elimination, monitoring, and control). If the management goal is to eliminate any new areas of CWD infection it is likely that smaller sample areas with

fewer animals will be needed to detect CWD infection before it becomes widespread in the population. For example, surveillance may be designed to have a high probability of detecting disease when prevalence exceeds 1% of the target population in a target region containing 200,000 free-ranging deer. With this design approximately 2,000 CWD infected animals would need to be present before the desired likelihood of detection was achieved. Current management information on CWD indicates that it would be extremely difficult, if not impossible to eliminate CWD after it has reached such a high frequency. In contrast, the population could be divided into a number of subunits (target regions), and surveillance could be designed to detect 1% prevalence in each of these units. For example, a target population with 20,000 free-range deer could then detect CWD when approximately 200 infected animals were present.

Of course, part of the practicality of disease control or elimination depends on the geographic spread and distribution of infected animals and the size of the target region; information that would typically be collected as part of a well-designed surveillance program for disease assessment. One alternative design would use a smaller target region containing fewer animals, so that CWD infection could be detected sufficiently early to increase the likelihood of eliminating the disease from the region. Another design would be to set a much smaller level for detecting disease prevalence within a larger area (e.g., 0.1% of 200,000 deer, or 200 CWD-positive animals). Either alternative would require greater sampling effort to achieve the surveillance design goals. At the present time, we don't know if it is feasible for even possible to eliminate CWD from free-ranging populations.

Sampling Frame and Selection of Sampling Units

In contrast to livestock disease surveillance, a wildlife target population is not easily identified for purposes of random or systematic sampling. The actual size and distribution of the wildlife population may not be known. And in most cases it is unlikely that a truly random sample of can be obtained from a population of free-ranging animals because all the individual animals in a target population cannot be identified for random selection.

To obtain statistically meaningful samples from wildlife populations may require dividing the target region or target population into smaller units to conduct efficient surveillance. In some cases, regions such as wildlife refuges or some tribal land units may be homogeneous or small enough that division into sampling units may not be required. In many situations the target region will be divided into sampling units (sub-regions or populations) that collectively constitute the sampling frame for that target region (see Appendix 2 for details). For example, if a state is the target region, sampling units might be counties, large game management units, or distinct animal populations. Collectively, these units will cover the entire state, or that portion of the state where free-ranging cervids are found. In other cases, sampling units might be defined by geographical characteristics on the landscape: roads, rivers, mountain ranges, or other features that provide physical or ecological barriers to animal distribution and movements. In the case of some populations, sample units might be biological groups

based on behavioral or physical environmental barriers. In the case of a population, it might be biological subgroupings of that population. The sampling units used may contain different numbers of animals or have different levels of CWD risk, factors that can influence the number of animals tested from each of the sampling units.

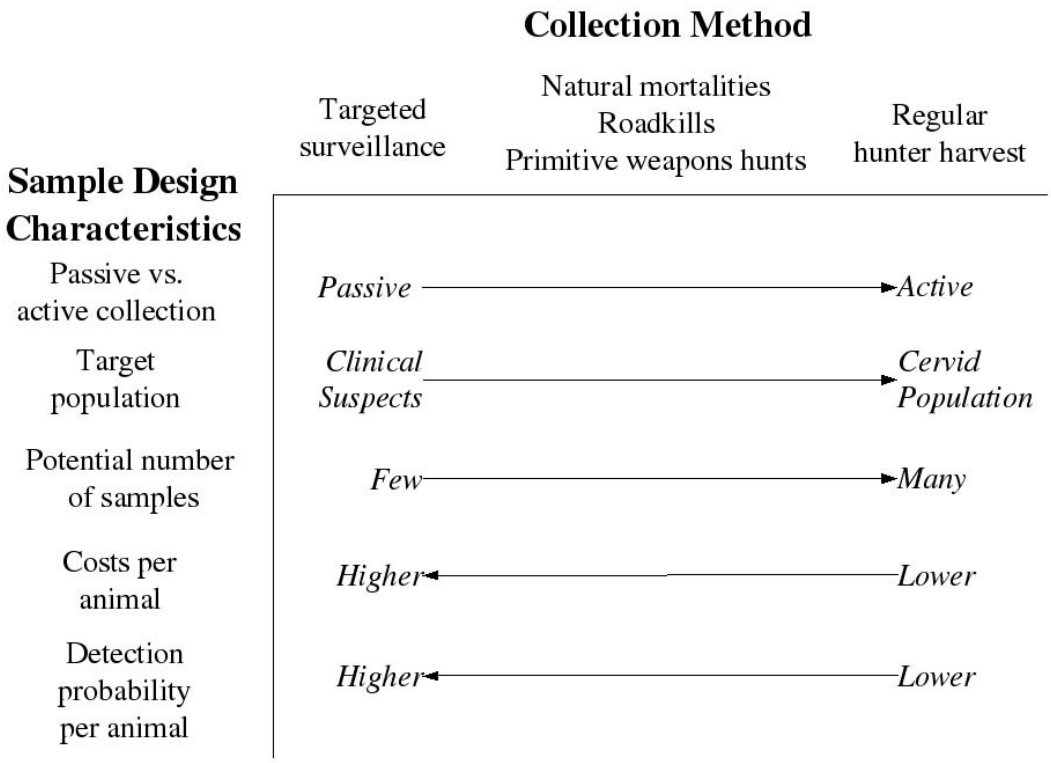
In most cases, elk and deer will not be evenly distributed throughout defined sampling units, nor is there an even distribution of risk factors for CWD. One method to address heterogeneous risk factors is to stratify the sampling units into groups with similar risk characteristics. For example, some counties within a state may have greater risk of CWD because they have a greater density of deer. For purposes of sampling design, the state could be stratified by county according to some criterion for deer density. Other risk factors, such as proximity to known CWD-positive animals in a neighboring jurisdiction or concentrations of game farms, also may be used. Establishing sampling units can help to ensure that sampling is distributed throughout the target region. Because it is generally desirable to detect CWD at low prevalence and because the disease is typically clustered on the landscape, it is important to ensure that samples are collected from throughout the target region (see Appendix 3). Determining how samples should be distributed from each of the sampling units in the target region can help to ensure the region is adequately sampled. For example, if a county is the target region, townships could be used to ensure that deer are collected throughout each county. The design could establish sample size goals for each township based on estimated deer density, to help ensure that deer throughout the county were collected in a representative (random) manner.

In practice, the distinction between target regions (target populations) and the delineation of sampling units within regions must be based on judgment and experience related to CWD risks, public perceptions, and management goals. Documenting reasons for selecting either the target region or sampling units will assist in analysis of results and help guide future assessments. How samples are drawn from the target population or region dictates what inferences may be drawn from the sampling results. This topic is discussed further under sampling considerations.

Collection Methods, Sample Design, and Sample Size

Methods for collecting free-ranging deer and elk, sampling animals for testing, and the number of samples needed are all key components of the surveillance strategy. Diagram Two presents a range of methods for collecting animals and how these methods might integrate with other aspects of the surveillance strategy. The importance of integrating the various aspects of a surveillance program including management goals, surveillance goals, and all the components of surveillance design cannot be overemphasized.

Diagram Two: Types of Sample Collection



1. Collection Methods

Surveillance methods for wildlife diseases are strongly influenced by the manner in which samples can be effectively collected. In contrast to livestock or other forms of disease surveillance, the wildlife population by definition is not easily manipulated for purposes of random or systematic sampling. At a more fundamental level, the actual size and distribution of the wildlife population itself may not be known. The collection method used to obtain animals for CWD surveillance will influence the sample design and number of samples that can be obtained. Type(s) of collection methods should be considered integral to the overall surveillance design.

Among the methods employed in CWD surveillance to date are the following:

Passive collection: Opportunistic sampling, such as the testing of road killed animals or collecting "sick looking" animals (targeted surveillance) relies upon chance that an animal will "present itself" for testing. In many circumstances it will be difficult to collect a sufficient number of sick, road-killed, or dead animals. Collection of deer and elk found dead from any cause can be submitted for CWD testing. At the present time, it is not clear if animals that have been road-killed or found dead have a greater or lesser probability of having CWD than the population as a whole. Symptomatic targeting or

targeted surveillance can be described as looking for (and testing) deer and elk that exhibit clinical signs consistent with CWD. Sample animals are selected on the basis of visual appearance and behavior. However, free-ranging cervids may also be affected by other diseases and health problems that cause them to exhibit clinical signs similar to CWD. Based upon experience in Colorado, Wyoming, and Wisconsin, as well as with farmed elk and deer, the probability of finding a CWD-positive animal may be greater among sick looking animals than the general population.

Active collection: Sampling from hunter-harvested deer is often the most practical and cost effective way to collect a large number of samples and may be relatively unbiased for sampling for CWD[12]. However, this method has certain drawbacks and obtaining accurate information from hunters is essential. Hunters often collect animals closer to roads or select certain classes of animals (e.g., large males), leaving more inaccessible areas or private lands where hunting is precluded as unsampled. Hunting regulations may affect the age/sex of harvested animals and seasons are generally short, unless extended for surveillance or management purposes. Hunters using primitive weapons may be more likely to shoot CWD infected animals as they may be able to get closer due to the behavioral changes of the diseased animal. Regulations, including sex or age restrictions, bag limit, season length and many other factors can affect hunter selection of animals from the population. Social and cultural factors, such as land ownership patterns and media coverage, influence the degree to which hunters support and participate in CWD management programs. These factors that may result in unequal probabilities of sampling deer, but hunters are essential for sampling on a large scale. Incentives for hunters to shoot unhealthy looking deer may be necessary if the hunter feels she or he would not eat venison from such an animal.

Sharpshooters also have been used in a number of sampling efforts, either to collect all the samples, to supplement samples from hunter kills, or to remove animals from infected areas. Such efforts are generally focused on limited areas and may be very costly compared with samples collected by hunters[13]. Depending upon the political climate, however, sharpshooters can be mobilized very quickly regardless of hunting season.

Live animal testing: The development of a live animal test for CWD in deer[14] permits sample collection, marking, and release of sampled animals, and selective elimination of CWD-positive animals. CWD-negative animals do not need to be killed, an important factor where public opposition to such killing would be strong. Live animal testing may be least disruptive to the elk and deer herds, but is likely only to be useful in limited areas

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[12] Conner, M. M., C. W. McCarty, and M. W. Miller. 2000. Detection of bias in harvest-based estimates of chronic wasting disease prevalence in mule deer. Journal of Wildlife Diseases 36: 691-699.

[13] The high cost may be more apparent than real. In the case of hunter kill collection, hunters heavily subsidize costs of collection, while other costs are spread out over time.

[14] Wild, M. A., T. R. Spraker, C. J. Sigurdson, K. I. O'Rourke, and M. W. Miller. 2002. Preclinical diagnosis of chronic wasting disease in captive mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) using tonsillar biopsy. Journal of General Virology 83: 2629-2634.
Wolfe, L. L., M. M. conner, T. H. Baker, V. J. Dreitz, K. P. Burnham, E. S. Williams, N. T. Hobbs, and M. W. Miller. 2002. Evaluation of antemortem sampling to estimate chronic wasting disease prevalence in free-ranging mule deer. Journal of Wildlife Management 66:564-573.

where low population density reduces the number of animals that need to be tested and where removal by other means is difficult (e.g., protected areas, parks, refuges). At $500 per animal or more, live animal testing is 5 to 6 times more expensive than collecting samples from hunter kills, and a level of skill is required to capture animals and collect samples. Because of the current delay between sample collection and test results, radio-telemetry is needed to locate and remove infected animals. New tests could reduce this delay and lower costs. Currently, live animal testing is only applicable to deer.

2. Sample Design

Sampling design refers to the manner in which the target regions (populations) are sampled, how sampling units are selected, and how collected animals are selected from the sampling units for testing. A variety of statistical approaches are available for selecting sampling units, sample sizes, and selecting animals for testing (Appendix 2). The manner in which sampling occurs determines the inferences that can be drawn from the results. The degree of confidence one may have that surveillance results can be inferred to the target region or target population is largely a function of the sample design, how well it represents the target population, and its execution. A more detailed presentation on sample design can be found in Appendix 2 and the associated statistical references. Because sample design and analysis can be complex, we recommend obtaining statistical expertise during the design and assessment of surveillance programs.

A wide variety of multi-stage sample designs are possible (see Appendix 2). In general, the more complex the objectives of surveillance, the more care (and expense) required in structuring the design and collecting animals. In some situations, such as targeted surveillance, all the animals collected may be tested as part of the design. When numerous hunter-killed animals are available it may be more appropriate to develop a specific sampling design to determine which animals to test. For example, detecting the presence of the disease in a small area might be addressed through opportunistic sampling, but understanding the epidemiology of CWD or determining prevalence rates in an already infected area would require a much more complex and rigorous approach. Rather than describing all possible sampling designs, this report illustrates the range of sampling designs and emphasizes the importance of sample collections that are as close to random as possible. A well-crafted design will have statistical validity and provide unbiased inferences about the target population.

Targeted sampling: In targeted sampling, wildlife managers collect deer and elk that show clinical signs of CWD. This approach is highly dependent on the capability of detecting and removing animals with clinical signs. If the goal of surveillance is detection, if risk of CWD is generally low, if resources are limited, and if hunter-killed deer are not available then this form of surveillance may be considered. The costs per sample may be greater for each animal collected, and only animals in the late stages of the disease will appear sick, meaning that the disease has probably been given time to establish itself within a population. This method may be less disruptive to the population as a whole, and additional diagnostic tests can provide other information regarding cervid diseases affecting the population. Unfortunately, the appropriate statistical inferences

from targeted surveillance depend on several untested assumptions (see Appendix 2). Thus, if the disease is not detected in the target population, we usually cannot determine the likelihood that CWD may occur in an area only that it is not present in the sick looking animals that have been collected. At the present time, the utility of surveillance based primarily on targeted animals is limited. Targeted surveillance should be used with caution until more information is available to determine the effectiveness of this method in CWD surveillance programs. This approach is currently recommended as a supplement to active methods of collecting animals (e.g., harvest) for testing or for very restricted situations with a high probability of detecting animals with clinical signs (e.g., areas with high human density).

Random sampling: Unlike targeted surveillance in which the sampling design is a function of clinically ill animals that present themselves, random sampling increases the confidence with which the results of surveillance can be extrapolated to the desired target population or region. Simple random sampling, for example, relies on creating an equal probability that any one animal in the target population (or region or its subunit) would be chosen for sampling. Stratified random sampling tries to ensure that each of the subunits (county, management unit) or animals are selected based on different probabilities that might be associated with disease prevalence or risk factors. Within each stratum, units or animals are typically selected randomly (Appendix 2).

Some form of random sampling is essential for following detection surveillance when the goals shift to determining prevalence and spread of CWD. It is also extremely helpful to conduct random sampling prior to detection of CWD because this can establish a baseline of data points, (albeit negative ones). This baseline allows one to create a context within which a single CWD-positive case, if found, can be evaluated. Besides the additional cost, however, random sampling in wildlife populations has practical limitations including the frequently unknown size of the whole population and the difficulty of achieving randomness over terrain that varies in accessibility. In some circumstances random sampling can be approximated across a target region by sampling animals in proportion to their abundance patterns or in proportion to habitat factors that are indicators of abundance.

Other sampling designs: Unequal probability random sampling permits the inclusion of risk factors into random sample (Appendix 2). This approach permits more intensive sampling in those areas where risk factors, such as a concentration of farmed elk or deer herds, are present. Adaptive cluster sampling, allows for a greater intensity of sampling in areas in which a CWD-positive case has been detected. This method is likely most useful after CWD has already been found and provides a potential mechanism for assessing local prevalence of disease in the area of a single CWD-positive case.

Selecting an appropriate sample design involves an ongoing assessment of goals, resource availability, human-dimensions issues, risk factors, and existing information on CWD in the target region or target population. States and federal lands that have experienced CWD have all gone through different stages of sampling designs as risk

factors and management goals have evolved. As experience with CWD grows, these designs will undergo further refinement and adaptation.

3. Sample Size

For any particular survey design, the number of random animals to be tested within a target population is a function of the sampling design and the degree of statistical confidence desired at a given hypothetical prevalence of disease (Appendices 2 and 4). Confidence levels are usually given in terms of percentage (99%, 95%, 90%, etc.) of surveys where disease would be detected ($\geq$ 1 CWD-positive animal) if prevalence were at least a certain percentage (1%, 2%, 5%, etc) of the target population. This confidence level represents the probability that at least 1 CWD-positive animal would be found if prevalence was at or greater than a specified level. Because of the association among confidence level, percentage of CWD prevalence, and sample size it is crucial to plan the surveillance program to collect the necessary number of samples for each target population and/or sampling unit. When samples size is insufficient, either the confidence level will be reduced or the prevalence that can be detected will be increased. For example, the results of a survey would be expressed as providing "95% confidence that CWD would be detected if the disease were present in $\geq$ 1% of the target population." Larger sample sizes are required to either increase the confidence level (e.g., 95% to 99%) or to decrease the assumed prevalence of disease (e.g., 5% to 1%) that could be detected. A smaller sample size might provide only "85% confidence that CWD does not exist at a level of at least 1% prevalence in the target population." It is important to realize there is a non-linear relationship among the sample size, confidence level, and detectable prevalence. For example, substantial increases in sample size may be required to move from a 90% to a 99% confidence level or to move from 2% to 1% detection of infection (Appendix 4).

Appendix 4 provides a standard framework for determining sample sizes required for different confidence intervals and different rates of infection when it can be assumed that individual animals are randomly chosen for testing. Such formulae should be applied with caution to surveillance in wild deer and elk because of the statistical assumptions required. Limitations include uncertainty regarding the size of the target population, the presence of disease clusters that are unevenly distributed throughout the population, and difficulties of collecting random samples of animals in the target population. Uncertainty in the size of the target population easily can be accommodated by assuming a target population level that is larger than expected or by assuming an infinite population (resulting in conservative estimates of the detection probability). In either case this will increase the sample size required to meet the specified confidence and prevalence levels.

More problematic are the assumptions that require a random distribution of disease and a random sample of animals from the target population. Current information indicates that CWD is not randomly distributed throughout free-ranging populations, but is distributed in disease clusters of infected animals. In addition, randomly sampling animals from any free-ranging population is challenging due to harvest regulations, hunter selection, land ownership and access, and other issues. Factors related to the distribution of disease and

sample collection can substantially influence the sample size required to achieve surveillance objectives and the inferences that can be made from the sample (Appendix 3). There are no simple solutions to these problems, but we offer several recommendations. First, select geographic sampling units to increase the chance that animals will be randomly sampled within each of these units (Appendix 2). These areas should be an appropriate size for detecting the occurrence of disease clusters of CWD, which is likely related to patterns of animal movement, and their distribution on the landscape. Second, use data on animal abundance or a relative measure like suitable habitat, to make an assessment of whether the sample distribution is similar to the distribution of animals in the target population. To help achieve a representative sample of animals from the target population collect a sample distribution that is related to the landscape distribution of animals.

Carefully consider how you will determine the appropriate prevalence to be detected. Scale this prevalence to the size of the target population and the management actions that will likely be implemented if CWD is detected. If the target population is very large (e.g., 500,000) then 1% prevalence would mean 5,000 infected animals. At this level of infection, it is likely that disease had been present in the population for some time and there will be many clusters of disease. Management options would likely be limited to no action, disease containment, or reduction in prevalence. In contrast, surveillance that divides large populations into much smaller target populations (e.g., 10,000) using 1% prevalence and 99% detection probability would likely detect disease in 100 animals, allowing management options that might eliminate or contain the disease. In some cases conducting surveillance with insufficient sample size to provide meaningful levels of detection probability (e.g., > 80%) and/or prevalence levels (e.g., < 5%) may be potentially misleading because little will be learned about the presence of CWD in the target population. Remember to consider the context of the target population and management goals when determining the desired prevalence for CWD detection.

4. Additional Considerations

Time period for sample collection: Conducting CWD surveillance activities over two or three years[15] within a target region may offer logistical advantages. During this period, sampling would be carried out in one area within a region or one of multiple populations in a jurisdiction the first year. In years two and three, survey the remaining areas or populations. Alternatively, a portion of the required sample size could be collected from each area in subsequent years. Distributing surveillance costs in this manner may allow for more intensive sampling than would be possible if the entire region were sampled each year. You could also conduct targeted surveillance across the whole region at the same time you randomly survey only part of the region.

At present there are no guidelines about how frequently to conduct follow-up CWD surveillance to detect new disease foci. Decisions about the frequency of follow-up

[15] The *incubation period* for CWD may be as long as 2-3 years, though wide variation exists. In addition, prevalence of CWD usually increases slowly. Leaving an area without surveillance for more than 3 years may increase the changes of the disease going undetected.

surveillance likely depend on the risk of disease occurring and on management goals. In high-risk areas where disease elimination is the management goal, some level of annual surveillance may be needed for early detection of new disease. In low-risk areas, sampling might be conducted at 3 to 5-year intervals, or with more frequent surveillance using opportunistic collection methods, such as targeted surveillance.

Data pooling: The greater the number of years over which CWD surveillance data is pooled, the fewer reliable inferences can be drawn. Data collected more than 3 to 4 years apart should not be pooled for analysis, because prevalence or even occurrence of disease may change over longer periods. Pooling, of course, may be less of a problem in jurisdictions with low risk of disease in which the disease has never been detected. However, data pooling over several years will be problematic if disease was introduced during the surveillance period. When surveillance is designed to assess prevalence or monitor changes in prevalence, pooling within 3 to 4-year intervals could introduce bias.

Test sensitivity and specificity: Sample sizes, such as those contained in Appendix 4, assume that the CWD test has 100% specificity and sensitivity. Currently, immunohistochemistry (IHC) using monoclonal antibody F99/97[16], is the gold standard; however, new tests are being developed. These tests may differ from the IHC test in specificity or sensitivity. If these tests (whose advantages may be reduced costs and faster reporting of results) come into widespread use, the numbers of samples taken in any particular survey should be adjusted in response to increased or decreased sensitivity or specificity. Frequently, the ability of current testing methods and sampled tissues to correctly diagnose infected animals is not considered. The CWD agent takes time to accumulate to detectable levels in animal tissues, and not all infected animals will be identified. In deer, it may take 4-6 months after infection before lymph and tonsil tissues become CWD-positive and these precede brain tissue. The pattern of disease progress is different in elk. Thus, sampling and interpretation of results should consider that recently infected animals will not be detected regardless of the diagnostic method, which will vary according to species and tissues sampled.

Age/sex stratification: Currently, we don't know whether prevalence of CWD differs in males and females within free-ranging elk and deer populations; although recent evidence from Colorado indicates higher prevalence in male mule deer. Given the long incubation period, we recommended that samples be collected from animals at least 1½ years old. This simple stratification by age may provide a greater probability of detecting the disease; preliminary information from Colorado, Wyoming, and Wisconsin indicates young animals are less likely to be infected long enough for disease to be detectable.

[16] Spraker, T. R., K. I. O'Rourke, A. Balachandran, R. R. Zink, B. A. Cummings, M. W. Miller, and B. E. Powers. 2002. Validation of monoclonal antibody F99/97.6.1 for immunohistochemical staining of brain and tonsil in mule deer (*Odocoileus hemionus*) with chronic wasting disease. Journal of Veterinary Diagnostic Investigation 14: 3-7.

III. Operational Activities and Costs of Surveillance

Operational activities of CWD surveillance should consider four interrelated processes and the human and material resources needed to run them: 1) internal and external communication, 2) sample collection, 3) diagnostics, and 4) data management. The components of these processes will vary based on the type of surveillance program implemented. As an example, Table Four outlines most of the major components involved in implementing a CWD surveillance program. This table was developed primarily for surveillance relying on hunter harvested animals as the main source of sample collection for testing.

Internal and External Communication

Because of media and public interest in this disease, the public agency (or agencies) charged with surveillance, especially if a CWD-positive case has been found in the jurisdiction, must be open with the public about its plans and findings, and what is known and unknown. Staff dedicated to communication and public information, or the use of public relations professionals, can be essential in easing the burden on scientific staff and optimizing information flow.

Internal and external communication of surveillance plans and findings are key components to this process. Local social, economic, and political factors should be considered when developing and communicating surveillance and management goals to the public. Public involvement in development of surveillance plans under the National Environmental Policy Act (NEPA) process may be required in some jurisdictions (e.g., federal agencies). Providing internal training, communicating logistical plans, and reporting results to staff are important components of internal communication. Training may be necessary for consistently detecting deer and elk with clinical signs of CWD, humanly collecting suspect animals, collecting tissues or other samples, processing and sample handling, and data management activities.

For surveillance using hunter harvests, communicating animal-specific test results to individual sportsmen is a particularly complex facet of this process that should be developed and managed by the responsible jurisdictions. General requirements may include instructions for sample submission, timeline for reporting results, objectives of the testing program, and interpretation of test results. It is important to emphasize that CWD test results are collected for management and research purposes and not as a food safety check.

A mechanism for collecting and disseminating CWD surveillance data to the public is highly recommended. Ideally, this information should be provided at several different

scales, including local, regional, and state or jurisdictional levels[17]. Until a national system is available, interagency and public access to surveillance results are desirable and will aid in assessing overall risks, management actions, and providing information to the public for making decisions about hunting opportunities and other factors..

Sample Collection

The system devised to gather and collect samples from animals is an important consideration; especially in communicating with hunters, landowners, meat processors, taxidermists, and agency personnel. Major elements of sample collection include clearly defined survey objectives, as well as logistical consideration of physical locations of the collection and laboratory sites needed to meet survey needs, tissue sampling strategies, transportation of samples, sample identification, sample storage, and disposal of carcasses and laboratory wastes. You should consider development of integrated computer systems to record, inventory, and track samples from the collection through testing processes. Staff training is required so that appropriate information and usable samples are consistently collected.

Targeted surveillance and live animal testing of deer may require additional staff training and planning considerations. Staff and volunteers should be trained to collect appropriate tissue samples from sampled animals. Alternatively, whole heads (or occasionally whole carcasses) may be submitted to diagnostic laboratories. Sample collection of tonsillar biopsies from live deer requires capture and anesthesia. Therefore, the associated cost is considerably higher than for post-mortem sampling.

Disposal costs for CWD-positive animals can greatly increase the overall expense of surveillance. These costs will vary, however, with the public's perception of risk involved in using landfills or other disposal methods such as incineration or alkaline digestion. Cost of carcass incineration varies, but can be estimated at $0.10 to $1.00 per pound. Alkaline digestion requires a large initial invested and maintenance costs of about $0.15 to $0.20 per pound. In areas where CWD has been detected, carcasses may need to be held until diagnostic test results are available so that appropriate disposition can be determined.

Total costs associated with CWD surveys can vary widely. Based on experience to date, it is assumed that CWD surveillance may cost anywhere from $50 to $100 per sample, exclusive of the cost of conducting the test. This cost can vary considerably depending of how animals are collected and requirements for disposal of infected animals. The National CWD Management Plan[18] has estimated a "national" average of $83 per sample for collection and testing from hunter kill surveys.

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[17] For an example see the Wisconsin Department of Natural Resources website: http://www.dnr.state.wi.us/org/land/wildlife/Whealth/issues/Cwd/results.htm

[18] Plan for Assisting States, Federal Agencies, and Tribes in Managing Chronic Wasting Disease in Wild and Captive Cervids. U. S. Department of the Interior and U. S. Department of Agriculture. June 26, 2002.

Diagnostics

An effective and efficient laboratory processing system for analyzing samples using a validated test is crucial. Generally, diagnostic samples need to be labeled, tracked, prepared, processed, evaluated, stored, and results reported. Although these are standard activities of most veterinary diagnostic laboratories, the volume of samples submitted from hunter harvest surveillance may tax the capacity and capability of most laboratories. Close coordination with the diagnostic laboratory is therefore essential. Capabilities, timeline for processing samples, and cost are a few of the items that should be discussed prior to submitting CWD surveillance samples. Submitters should consider using a USDA certified CWD testing laboratories.

Several laboratory tests can be used to diagnose CWD and additional assay development is currently underway. The current gold standard diagnostic test is immunohistochemical staining (IHC) using MAb 99/97.6.1[19] on brain, retropharyngeal lymph node, or tonsil. Other screening tests using ELISA methods are also being developed for use on these tissues. At the present time, positive screening tests should be confirmed using IHC, especially when confirming presence of CWD in a new area. Prior to determining which diagnostic test to employ, it is advisable to assure that the test has been validated for sensitivity and specificity in the tissue and the species for which it will be used. Costs of tests vary with type and laboratory, but are generally $15 to $25 per sample. Other considerations for the selection of assay methods include time required to process samples, the volume of samples that can be processed, and the condition of the tissues (e.g., freshness or decomposition) that will be that will be tested.

Data Management

Integral to all the other processes is a system of data collection and management that links the entire surveillance operation providing biological and epidemiological information for disease management as well as public information. This includes sample collection information, tissue tracking, inventory, and test results.

Collection of raw data on samples may be electronic (e.g., PDA system used by Michigan Department of Natural Resources) or hard-copy that is later transferred to electronic format. Marking samples with bar codes provides an efficient system for electronically tracking samples through the testing process. Although electronic systems require upfront cost for system development and equipment purchase, savings may result from increase efficiency.

[19] Spraker, T. R., R. R. Zink, B. A. Cummings, M. A. Wild, M. W. Miller, and K. I. O'Rourke. 2002. Comparison of histological lesions and immunohistochemical staining of proteinase resistant prion protein in a naturally-occuring spongiform encephalopahty of free-ranging mule deer (Odocoileus hemionus) with those of chronic wasting disease of captive mule deer. Veterinary Pathology 39:110-119.

To assess how samples are being drawn from the target population, an accurate record of location of animal collection is required. Methods for facilitating accurate hunter kill locations need to be developed for each area. UTM coordinates may be useful for this purpose. Determining the best measurement scale for recording animal collection locations (e.g., UTM, land parcel, section, township, management unit) depends on the survey design, size of the area used by animals, movement patterns, landscape patterns, and surveillance objectives. Animal locations should be collected at a scale that provides the most accurate results that can be reliably achieved; location data can later be summarized at scales with less resolution if warranted.

Coordination between jurisdictions collecting data may lead to a national CWD database in the future. However, currently no integrated system for CWD surveillance is available.

Table Four. Components of CWD Surveillance

Component	Description
Internal and external communication	Communicate surveillance goals, objectives, modifications of protocols and procedures as new information becomes available. Consider local social, economic, and political factors to develop and communicate a public message regarding the management and surveillance goals. Use skilled public relations professionals to keep the public informed.
Set up and manage sample collection system, including disposal	Includes field stations and transportation, with multiple logistical elements dictated by survey objectives and local needs.
Set up and manage lab processing	Includes all steps necessary to obtain targeted tissues, provision of space, supplies, and storage room
Set up and manage data processing	Includes collecting and managing all field and laboratory data relevant to sample collection, tissue tracking, obtaining, and posting results.
Human Resources	Staffing and training employees and, if used, volunteers to implement surveillance and management goals.
Procurement of supplies	Obtaining of materials needed for the above components.

IV. Research Needs for Improved Surveillance

To date, surveillance for CWD in free-ranging cervids has primarily been limited to jurisdictions (primarily states and provinces) where the disease has already been found.

Because of this, our knowledge about the geographic distribution, prevalence, and occurrence of CWD in natural populations and habitats is limited. The need for increased surveillance was identified as one of the important components of a national plan for CWD. However, collecting and testing animals for an extensive surveillance program would likely require millions of dollars. More effective surveillance programs will require additional research on CWD to understand the epidemiology of the disease, develop statistical and simulation models to improve our surveillance programs, identify disease risk factors, and develop statistical theory and methods. Because of the importance and magnitude of surveillance programs to CWD management, we have identified several areas in which additional research would improve the current approaches being taken to CWD surveillance.

Epidemiology and Modeling: The most fundamental research need is to better understand the epidemiology of this disease. Many components of the surveillance program (including the selection of surveillance methodology, methods for collecting animals, frequency of conducting surveillance programs, risk factors, and distribution of sampling effort) depend heavily upon an understanding of how CWD is transmitted among animals and across the landscape. New and better knowledge of animal behavior, population characteristics, and dynamics relative to the occurrence and spread of CWD would be important to developing this understanding. The creation of accurate models of how CWD spreads across the landscape, in spatial-temporal dimensions, requires further exploration and has direct application for design of surveillance programs. Research is underway to create both statistical and simulation models, but these are in an early stage of development. This modeling effort will allow us to ask "What if" questions to help provide guidance for both surveillance and management goals, and enhance our ability to design and implement more efficient and cost effective surveillance methods. Additional work in this area is crucial for a more complete understanding of CWD surveillance, and the long-term effects of this disease on elk and deer populations in the United States.

Identify Risk Factors: At the present time, our knowledge of the risk factors associated with the exposure and amplification of CWD (Table Two) is based primarily on general information about similar infectious disease processes, general knowledge about deer and elk biology, judgment about the origins of the disease, and limited observations of disease spread. Better information to identify and quantify risk factors would help guide decisions on surveillance priorities and allocation of resources to conduct surveillance programs. As our ability to determine risk factors associated with exposure and amplification of CWD improves, surveillance methods (as well as management techniques) will improve.

Statistical Methodology: New or modified statistical methods could greatly increase the efficiency of CWD surveillance programs. In particular, statistical methods and guidelines to effectively detect diseases that occur at low prevalence, are locally clustered, and are uncommon at large scales (e.g., states) are lacking. One method of collecting animals, targeted surveillance based on animals with clinical signs, holds considerable potential for improving the efficacy of surveillance, especially for detection of CWD-positive cases. However, the sampling framework and interpretation of results

from this method requires further investigation in several areas (Appendix 2). The rigor with which "sick looking" animals can be observed, consistently identified, and collected is unknown. Statistically interpreting the results of targeted surveillance where no CWD cases are found causes difficulties for estimating the likelihood that CWD is below a specified prevalence. In addition, understanding the ways in which CWD-positive samples could be extrapolated to the target population of "sick" animals and then to the whole population would help to estimate the prevalence of disease. We need to develop statistical methods that allow the combination of several different collection methods, with potentially different biases, into combined statistical estimates that could be used to determine CWD prevalence, likelihood of CWD being present, and other statistics of interest.

Cervid Biology and Hunting: In a number of areas, ungulate research could improve our surveillance for CWD. A better understanding of the factors that influence seasonal movements of animals, the patchy distribution of CWD, and interactions of cervids could provide more management options for reducing the spread of CWD, better information for determining target populations, and improvement in strategies for surveillance. In addition, we know even less about hunter and landowner behavior and harvest patterns. If CWD control or elimination is a management goal, a better understanding of hunter motivations and behavior is required.

Because little is known about epidemiology and distribution of CWD in free-ranging cervids, we need to develop effective surveillance strategies based on identified risk factors, enhance early detection, and support management and research programs. Surveillance strategies should play a key role in CWD management by identifying the presence of disease, determining the distribution and prevalence of disease across the landscape, and by monitoring disease trends or the effects on management actions to control CWD. Surveillance activities are also needed to satisfy public and management information concerns about the location and prevalence of disease in different geographic areas. Surveillance programs are a fundamental component of any CWD management program and surveillance should be considered as an integrated component of any research, management, or monitoring strategies.

Glossary

Assessment	The process of determining the spatial extent, spatial distribution, and prevalence of CWD in an area. The second stage of surveillance, applicable in areas where CWD is newly identified.
Clinical signs	Presentation of observable signs of disease. In CWD, these include loss of body condition, behavioral changes including a blank stare, excessive salivation, excessive drinking and urination, depression, and lack of coordination.
CWD-negative	State where PrP^{CWD} cannot be detected in an individual using immunohistochemistry of lymphoid, tonsil, or brain tissue.
CWD-positive	State where an individual is considered to harbor the proteinaceous infectious particle associated with chronic wasting disease (PrP^{CWD}), based on the results of a validated test for PrP^{CWD} including immunohistochemical or other immunologic test such as ELISA and Western blotting. Note that due to the long incubation period associated with CWD, a CWD-positive individual may not show clinical signs. Further, there appears to be a period of unknown length where an individual that harbors PrP^{CWD} will test CWD-negative with current testing methods.
Detection	The first stage of CWD surveillance, applicable in areas where CWD has not previously been described.
Disease Cluster	A group of animals in which CWD prevalence is elevated relative to other groups of individuals or to an expected background level. Disease clusters are commonly thought of as spatially defined (e.g., a particular area may have higher prevalence than surrounding areas); however a disease cluster may be defined by biological factors such as membership in a herd.
Gold Standard	Refers to a diagnostic test that is considered the best available indicator of the presence or absence of a pathogen.
Immunohistochemistry	Commonly referred to as IHC, a diagnostic technique that uses immunological staining of brain, tonsil, or lymphoid tissue to detect the presence of PrP^{CWD}. Currently IHC is considered the gold standard test for the presence of PrP^{CWD}.

Incubation period	The period of time from initial infection with PrP^{CWD} and the development of clinical signs. Incubation period is often incorrectly used synonymously with "latency", which refers to the time between first infection and infectiousness.
Latent period	The period of time from initial infection with PrP^{CWD} and the time at which the affected individual is capable of transmitting PrP^{CWD} to other individuals.
Monitor	The third stage of surveillance, applicable in areas where CWD has been identified and the objective is to determine trends in spatial extent, prevalence, response to specific management actions and other factors. CWD monitoring should be closely linked to management or research objectives.
Prion disease	Transmissible spongiform encephalopathy characterized by accumulation of PrP^{CWD}. Disease is believed caused by an aberrant protein rather than by a virus, bacteria, or other pathogen that contains nucleic acids.
Prevalence	An estimate of the proportion of the target population that is CWD-CWD-positive based on a sample of the population. Prevalence is often estimated by the number of individuals that test CWD-positive for CWD divided by the number of individuals for which there are valid test results. Because of the clustered nature of CWD, reports of prevalence should be accompanied by an explicit statement of the number of samples tested and the spatial area from which the samples come. In addition to spatial patterns in prevalence, CWD prevalence may vary among sex and age classes.
Random sampling	Where each animal in the sampling unit has an equal probability of being sampled. Most statistical methods rely on random samples being obtained from a target population; for free-ranging animals achieving this goal is typically very difficult.
Risk factor	Factor associated with an increase in the probability of an individual being CWD-positive. Factors can be divided into two groups: 1) exposure risk factors that may increase the probability that CWD will be introduced to a population and 2) amplification risk factors that may increase the rate of spread through a population.

Sample design	Detailed description of the objectives, target population, sampling units, and methodology used to obtain a sample of the cervid population for CWD testing. See Appendix 2.
Sampling	The process of selecting individuals from a target population in order to make inferences about the target population. See Appendix 2 for a discussion of different sampling methods.
Sampling frame	A list of the sampling units from which samples of animals will be taken for CWD testing. See Appendix 2 for details.
Sampling unit	Mutually exclusive portions of the target population that cover the entire population. Sampling units could be delineated by biological characteristics such as herds or subpopulations, or by administrative boundaries such as townships, management units, or counties. Commonly, sampling units are chosen at a scale such that each individual in the sampling unit is equally likely of being sampled and that meets management surveillance objectives.
Sensitivity	Ability of a diagnostic test to identify individuals that harbor PrP^{CWD}. Estimated by the proportion of individuals harboring PrP^{CWD} that test CWD-positive on a particular diagnostic test. See specificity.
Specificity	Ability of a diagnostic test to correctly identify individuals that do not harbor the proteinaceous infectious particle associated with chronic wasting disease (PrP^{CWD}). Estimated by the proportion of individuals not harboring (PrP^{CWD}) that test CWD-negative on a particular diagnostic test. See sensitivity.
Target Population	A group of individuals that are spatially, genetically, or demographically distinct from other groups about which inferences are to be made. In practice, target populations may be defined by political or administrative boundaries or may represent distinct groups of animals where interchange is limited.
Target region	A geographical region (and the animal population contained therein) about which we wish to make a statistical inference.
Targeted surveillance	Surveillance approach in which wildlife managers are encouraged to collect deer and elk with clinical signs of CWD for testing.

Appendix 1 - National Surveillance for Chronic Wasting Disease: A Technical Workshop

Workshop Purpose and Background:

This workshop will focus on designing, developing, and implementing CWD surveillance programs for free-ranging cervids. Surveillance programs are being carried out under a wide range of variables including: surveillance goals, landscapes, population characteristics and dynamics, regulatory frameworks, species (elk, mule deer, white-tailed deer), and available resources. Surveillance programs, at the same time, have to build in the most appropriate science in terms of sampling, surveillance methodologies, transmission models, and assessment of results. There is a great need for systematic guidelines that state, federal and tribal agencies should follow in developing surveillance programs.

Objectives:

1) Define the surveillance goals for management of CWD,
2) Establish the key operational and logistics components for conducting a surveillance program,
3) Develop prototype statistical methodologies and procedures for CWD surveillance (detection, distribution, and monitoring), and
4) Determine the direction for research on CWD surveillance.

Outcomes:

Products from this meeting will include a white paper that reviews the current state of knowledge on planning, conducting, and evaluating a CWD surveillance program. The audience for this paper will include state, federal, and tribal agencies carrying out CWD surveillance in wild cervid populations. The white paper will address the following areas:

1) Evaluate alternative management goals for CWD surveillance in wild cervids (disease detection, disease distribution, disease monitoring),
2) Review and describe key operational considerations for planning and conducting CWD surveillance (collecting, processing, disposal, testing, inventory, etc.),
3) Review and discuss statistical guidelines to meet CWD surveillance objectives (number of animals, distribution of samples, years sampled, source of animals {clinical suspects, road kills, live sampling, hunter harvest, etc.}, analysis and interpretation), and
4) Develop a list of research needs.

Appendix 2 - Sampling Designs for Surveillance

The objective of detection surveillance is to detect one or more CWD infected deer (or elk) in a target region(s) with a specified probability, given an assumed level of infection (prevalence) in the target population. More specifically, we wish to state "if the prevalence of CWD in animals in the target region/target population is at least [x]%, then we are [y]% confident that our surveillance plan will detect at least one infected animal." The validity of such a statistical inference depends on an explicit description and use of a statistical sampling design and assumptions about the sampling process.

The discussion and examples provided here are intended to serve as a framework for planning and implementing surveillance designs. However, every situation will be different, with its own particular set of logistical and financial constraints, and our objective is thus to provide a general framework that can be adapted to specific situations. In addition, implementation of any design will involve many practical compromises -- such is the nature of any field study of free-ranging wildlife populations. Due to realist constraints, the best design is not always feasible. Nevertheless, we believe that the structured thought process described here is essential in developing any surveillance design, because it emphasizes the importance of explicit definitions of the statistical objectives, the methodology used to achieve the objectives, clear definition of the target population and sampling units, and acknowledgement of the assumptions required for valid statistical inference.

We currently have limited information about the epidemiology of CWD in free-ranging cervid populations, including spatial distribution, prevalence, and temporal dynamics of the disease. Understanding of the disease in white-tailed deer in the central and eastern United States is especially limited. Lacking such information, we cannot realistically evaluate the efficiency of alternative sampling designs, and the statistical consequences of violation of assumptions such as random sampling of animals. We expect that surveillance designs will markedly improve as additional information from field studies becomes available, and computer simulation studies are conducted of the statistical properties of sampling designs under different sets of realistic assumptions (see section on Research Needs).

There is a voluminous literature on theory and application of finite population sampling designs, and a myriad of design choices[20]. We briefly describe a few designs that seem most applicable to CWD surveillance. We assume a conceptual multi-stage sampling frame that is hierarchical and thus involves multiple layers of sampling units. At each stage, choices are made about where and how to select sampling units. For simplicity, we consider only two-stage or three-stage designs. For all stages, two basic elements are required: 1) definition of the structure of the sampling frame, 2) the method of random selection of the samples.

[20] Cochran, W. G. 1977. Sampling techniques. 3rd ed. Wiley: New York, New York.
Thompson, S. K. 1992. Sampling. John Wiley & Sons: New York, New York.

Sampling Geographic Units: Conceptually, the target region can be structured as a collection of primary sampling units (PSUs), and within each PSU, several secondary sampling units (SSUs). First, we consider a stratified design. Stratification typically involves partitioning the target region into different geographic strata or by categorical characteristics (e.g., animal abundance levels, disease risk levels) associated with groups of sampling units. A sample of units will subsequently be selected from every stratum using a design created for the second stage. For example, if the target region is a state, strata might be administrative wildlife management units, counties, or physiographic regions that are divided into categories associated with suspected level of disease risk (e.g., high, moderate, low). On a smaller scale, if the target region is a county, the strata could be townships.

Alternatively, we might choose a design with unequal probabilities constructed using relevant ancillary information. As an example, consider a design in which counties are the PSUs and the entire state is the target region. We might wish to sample only a relatively small number of counties, but we want a greater chance of randomly choosing those counties that contain larger numbers of game farms (proportional sampling), which might be considered a risk factor for the presence of CWD. We would calculate the proportion of the state's game farms that occur in each county (PSU), and use these proportions as selection probability for each county. In this design, the chance of selecting a county with five times as many game farms as another county will be five times greater.

Sampling Animals: If the target region has been stratified, then several choices exist for determining how and where to sample animals within strata.

The simplest example is when harvested animals are collected at check stations or road kills are collected opportunistically. In this case, the conceptual sampling frame consists of a target population of animals within a defined region, and it is assumed that the harvested or collected sample of animals represent an equal probability (random) sample. If the strata are relatively large and the number of check stations or collection points is small or spatially clustered within the stratum, then the assumption of a random sample is probably invalid because not all animals in the target population will have an equal probability of being sampled and therefore the statistical validity of the design is compromised. This problem could be improved by placing additional structure on the surveillance design. For example, contiguous blocks of four townships within a stratum could be considered PSUs, and a sample of these selected at random. Animals are collected within these PSUs. In practice, the animals may be collected using check stations, but now the assumption is that these animals are a random sample from the township block as opposed to the entire stratum. This approach would be expected to be statistically valid if the disease is spatially clustered at a relatively small scale within the stratum. Note that the complete design in this example has three nested stages: strata (counties), PSUs (township blocks), and SSUs (individual animals within PSUs).

If the target region is partitioned into PSUs at the first stage, then the next task is to define the SSUs. For example, if PSUs are counties and the target region is a state, then SSUs could conceptually be defined as the population of animals within the county, and a sample of individuals that are collected from the county by some means that should be designed to attain a random sample. As before, this assumption of randomness is likely to be violated depending on the spatial distribution of the disease and the distribution of animals sampled within the county (Appendix 3). One strategy to help achieve a random sample would be to define smaller SSUs such as townships. This may facilitate spatial distribution of the samples within the county (PSU) according to factors such as deer abundance or deer habitat. In addition, random testing of animals collected from a township may also facilitate a random sample of animals from within these townships. Note that again in this example, the complete design involves 3 conceptual nested stages.

As a second example, suppose that the target region is a single contiguous geographic area that is chosen because it is deemed to have the greatest risk of infection. A list of meat lockers that process harvested animals is available for the target region, and it is assumed that the animals brought in for processing represent a random sample of the target population within some definable area within the region. We could then define the meat lockers as the PSUs, and the individual animals within a locker as representing the SUs. A sample of lockers could be chosen using some sampling scheme, and then a sample of animals can be chosen at random from the locker.

Targeted Surveillance: Targeted surveillance is one strategy for detecting CWD in a target region. This approach is based on the reporting, removal, and testing of animals that exhibit clinical signs of CWD infection in the field. This strategy assumes that criteria for determination of clinical signs (hereafter clinical) are clearly and precisely stated and uniformly applied in the field. In addition, this strategy is likely to be most effective when there is a high probability that animals with clinical signs will be reported and removed from the population. Clearly, this strategy does not result in a random sample of the entire population in the target region, and thus probabilistic and statistical statements about detection rates, prevalence rates, and confidence levels may not be possible. However, given additional information and some assumptions, statistical interpretation of these results is possible.

Assume that the sample of clinical animals from targeted surveillance represents a random sample from the subpopulation of clinical animals within the entire population. Then the proportion of this sample that is infected with CWD (α) is an unbiased estimate of the CWD prevalence rate in the clinical subpopulation. Next, assume that a preliminary survey of the entire population (healthy and clinical) is made to estimate the proportion of the entire population that is clinical (ψ). Finally, assume a value, presumably obtained from the literature or empirical data, for the proportion of CWD infected animals that exhibit clinical signs of the disease (β). Then an unbiased estimate (θ) of CWD prevalence in the entire population within the target region is $\theta = \alpha (\psi/\beta)$.

The implication of this relationship for a detection strategy based on targeted surveillance is revealed by re-expressing the relationship as $\alpha = \theta (\beta/\psi)$. Thus, the CWD prevalence

rate in the clinical population is greater than the CWD prevalence in the entire population by the factor (β/ψ), the ratio of the proportion of the CWD population that is clinical to the proportion of the entire population that is clinical. The statistical efficiency gained with this type of detection strategy thus depends on this ratio.

A simple example will help to illustrate this application of the method. Suppose we adopt the "99 – 1" objective of being 99% confident of detecting CWD in a target population with at least 1% prevalence. Then, assuming simple random sampling, we know that a sample size of 458 animals is necessary. However, now assume that 10% of the animals infected with CWD are clinical, and that data from a field survey has produced an estimate of 3% for the proportion of the entire population that is clinical. The "effective" prevalence rate that we are now sampling = 1% (10% / 3%) = 3.33%, and we need 137 clinical animals to achieve the same 99% confidence level.

Although these results illustrate that targeted surveillance might be more efficient than designs based on sampling the entire population, we must emphasize that additional data and assumptions are necessary to implement the method. In particular, information on the proportion of clinical animals found in the target population and the proportion of CWD infected animals that exhibit clinical signs may be problematic. These parameters may be difficult to acquire for wildlife populations; may change seasonally, annually, or geographically; and may be affected by other disease or health problems affecting the population. In addition, it may be extremely difficult to obtain the required number of clinical animals for a meaningful target population (e.g., county or DMU). Further research is needed to address these and other issues related to clinical surveillance strategies (see Research Needs section).

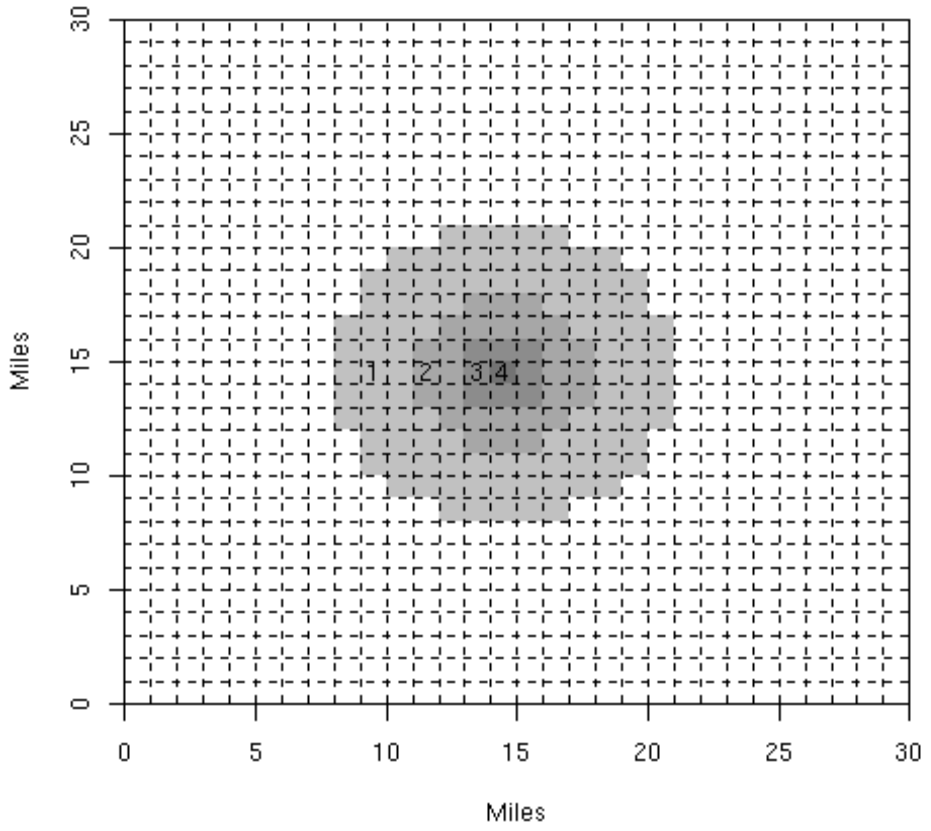
Appendix 3 - Effect of Spatial Distribution of CWD and Sampling Locations on Detection Probability

An important consideration in the design and interpretation of CWD surveillance activities is determining the target population for which inferences are to be made. Statistical methods usually assume the entire target population is "randomly sampled." Specifically, each individual in the population is assumed to be equally likely to be CWD-positive as well as equally likely to be tested for CWD. However, several epidemiological and ecological factors likely cause violation of this assumption. As with most newly emerging infectious or transmissible diseases in wildlife, CWD is unlikely to be distributed randomly through a population; there will be areas of greater and lesser prevalence. Further, variation in hunter access, terrain, and human population densities among other factors will likely result in spatial variation in sampling effort.

A computer simulation was used to illustrate how spatial aggregation in disease prevalence and sampling effort can reduce the probability of detecting CWD from the idealized situation where CWD is randomly distributed and is randomly sampled. Specifically, we examined how the probability of detecting at least one CWD-positive individual is affected by sample size and spatial autocorrelation in sampling effort. For simplicity, simulations assumed that hunters collected CWD samples.

Simulations were based on sampling for CWD in white-tailed deer within a simplified landscape using features typical to habitat east of the Mississippi River. Deer density was uniform across a 30 by 30 mile (48 by 48 km) landscape with 20 deer in each of 900 one-square-mile cells (i.e., 20 deer per mile^2 or 7.7 deer per km^2, totaling 18,000 deer). Based on current understanding of CWD distribution in Wisconsin, we assumed CWD prevalence was greatest in a disease cluster at the center of the landscape, and declined with distance from the center of the disease cluster (Appendix 3: Figure 1).

Prevalence at the core of the disease cluster was 20% (4 CWD-positive of 20 deer in the center cell), and declined from the center (3, 2, and 1 CWD-positive of 20 deer per cell respectively). Total prevalence on the simulated landscape was approximately 1% (184 of 18,000 deer). Based on some assumptions about CWD epidemiology (18 month latent period, a 36 month life expectancy after infection, and transmission from each CWD-positive deer to 2.5 to 3 other deer), about 170 CWD-positive deer could be present within 3 to 4 years after introduction of a single CWD-positive individual to a CWD-free population.



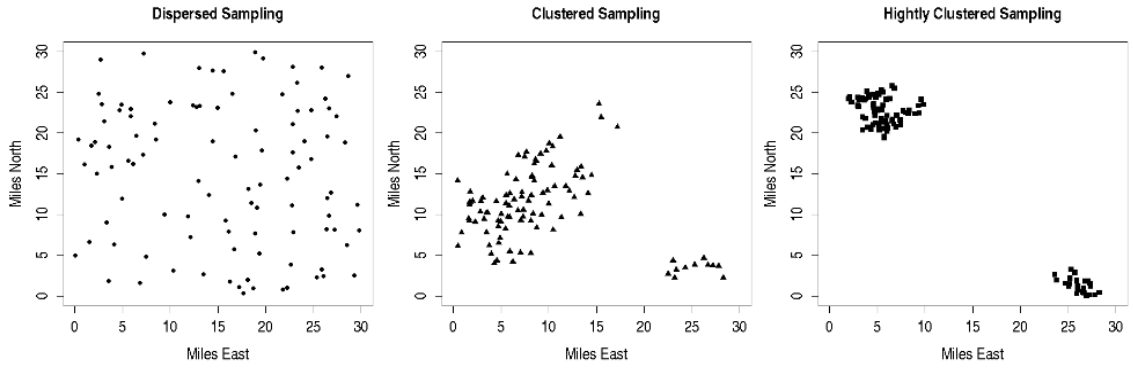
Appendix 3: Figure 1. Landscape used in sampling simulations. Each square mile cell was assumed to hold 20 deer, with a total of 18,000 deer. Numbers in the shaded cells indicate the number of CWD-positive deer in each cell with the same shade.

We used computer simulation to investigate the probability of detecting CWD based on different sample sizes (50-500) and different spatial distributions of samples (Appendix 3: Figure 2). Simulations were repeated 100 times at each sample size and spatial distribution to estimate the probability of detecting CWD in a landscape. Hunters are unlikely to sample deer populations at random because roads, land access, human density, and other factors create some degree of clustering where deer are shot. This aggregation would likely result in areas where the deer population is over-represented in the sample and other areas where there is a sampling deficit of sampling.

We simulated the non-random nature of samples obtained from hunter-killed deer by using three levels of spatial autocorrelation as illustrated in Appendix 3: Figure 2. These aggregation patterns included:

1) "dispersed" sampling with a small degree of autocorrelation such that most of the landscape was sampled,

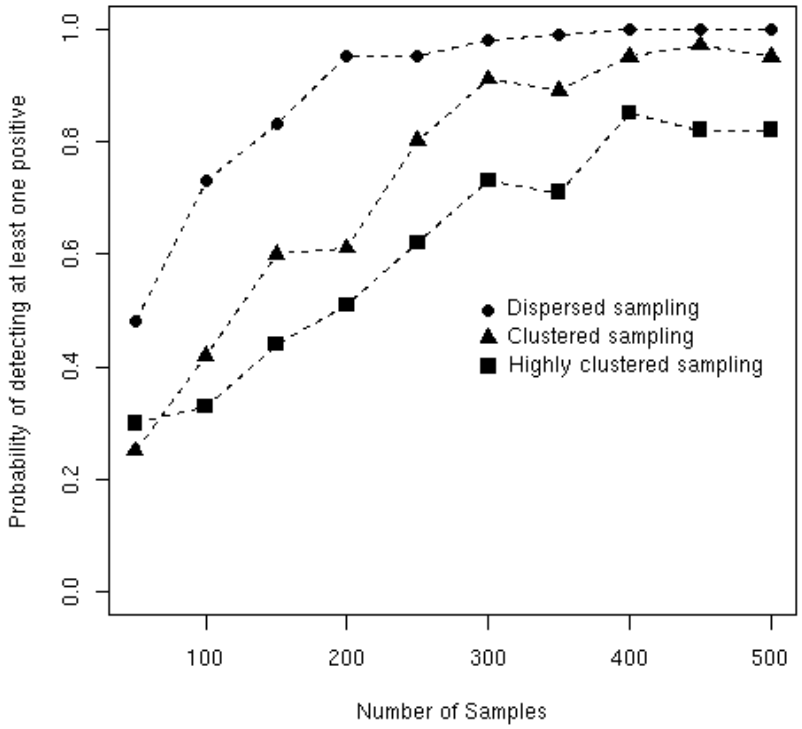
2) "clustered" sampling with a moderate degree of spatial autocorrelation in sampling effort, and
3) "highly clustered" sampling with a high degree of spatial autocorrelation.



Appendix 3: Figure 2. Examples of distributions of sampling locations used in the simulations. For each set of simulations, samples were taken in a distribution that was dispersed (left panel), clustered (center panel) or highly clustered (right panel). These panels correspond with the probability curves shown in Appendix 3: Figure 3, matched by common symbol.

The probability of detecting at least one CWD-positive deer was strongly correlated with both degree of aggregation in sampling locations and number of samples (Appendix 3: Figure 3). The estimated detection probability from simulations converged on that predicted by random sampling when sample sizes were large (e.g., >300 deer) and when sampling was evenly distributed across the landscape. However, for spatially aggregated samples the probability of detecting at least one CWD-positive deer was considerably below that expected from a truly random sample.

These results emphasize the importance of randomly sampling from deer populations to achieve the expected detection probability (Appendix 4). Local variation in deer densities, age- and sex-specific variation in CWD prevalence, hunter behavior and other factors also may affect the probability of detecting CWD. However, the general pattern illustrated will likely remain true; sampling effort must be well distributed throughout the target population to minimize the possibility of missing disease clusters. In addition, appropriate stratification of sampling effort with respect to prevalence of CWD (or suspected risk factors) will increase the probability of detection of CWD.



Appendix 3: Figure 3. The probability of detecting CWD on a landscape as a function of sample size and dispersion of samples.

Appendix 4 - Sample Size Required for Detecting CWD with Random Sampling

This table provides two types of information:

1) the sample size (cells) necessary to detect at least one CWD-positive animal in a target population of known size (rows) with a specified disease prevalence (italicized columns); and
2) the number of CWD-positive animals that could be present (cells) without disease being detected, given a specific population size and specific number of CWD-negative results.

To determine the sample size (cells) required for a specific problem, choose the detection probability (top column), target population size (rows), and the desired detection limit for prevalence (italicized columns). For example, if the management goal is a 90% probability of detecting CWD at a prevalence of 1% or greater in a target population of 1500 animals, 213 samples are necessary (see shaded cell).

To determine how prevalent CWD could be in a target population from a given number of CWD-negative results, choose the detection probability (top column), target population size (rows), and the proportion of the target population that has been sampled (italicized columns). For example, if 1% of a target population of 1500 were tested (15 samples), there would be a 90% probability of detecting at least one CWD-positive if 14% (213/1500) of the deer in the target population were CWD-positive.

This table makes the following assumptions:

1) random sampling is conducted so that each individual in the target population has an equal likelihood of being sampled. Violation of this assumption is discussed in Appendix 3.
2) test sensitivity and specificity are each 100%.

Probability of detecting one positive:	90%					95%					99%				
Prevalence, **or** Percent of Population Sampled	*10%*	*5%*	*2%*	*1%*	*0.1%*	*10%*	*5%*	*2%*	*1%*	*0.1%*	*10%*	*5%*	*2%*	*1%*	*0.1%*
Population Size															
10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
50	19	30	46	50	50	22	35	48	50	50	30	42	50	50	50
100	21	37	69	91	100	26	45	78	96	100	36	60	90	100	100
150	21	40	81	118	150	27	49	95	130	150	39	68	118	143	150
200	22	41	88	137	200	27	51	105	156	200	40	73	136	180	200
300	22	43	96	161	300	28	54	118	190	300	42	78	160	235	300
400	22	43	100	175	400	28	55	125	211	400	42	81	174	273	400
500	22	44	103	185	496	29	56	129	225	500	43	83	184	300	500
1000	23	45	109	206	901	29	58	139	259	951	44	87	205	368	991
1500	23	45	111	213	1177	29	58	142	271	1297	44	88	212	396	1431
2000	23	45	112	217	1368	29	59	144	278	1553	44	89	216	410	1800
2500	23	45	112	220	1505	29	59	145	282	1746	44	89	219	419	2104
3000	23	46	113	222	1608	29	59	146	285	1895	44	89	220	426	2353
3500	23	46	113	223	1687	29	59	146	287	2013	44	90	222	430	2561
4000	23	46	113	224	1751	29	59	147	288	2108	44	90	223	434	2735
4500	23	46	114	224	1802	29	59	147	289	2187	45	90	223	437	2882
5000	23	46	114	225	1845	29	59	147	290	2253	45	90	224	439	3009
6000	23	46	114	226	1912	29	59	147	292	2358	45	90	225	442	3214
7000	23	46	114	226	1962	29	59	148	293	2437	45	90	225	444	3374
8000	23	46	114	227	2001	29	59	148	294	2499	45	90	226	446	3501
9000	23	46	114	227	2032	29	59	148	294	2548	45	90	226	448	3604
10000	23	46	114	227	2057	29	59	148	295	2588	45	90	226	449	3690
50000	23	46	115	230	2250	29	59	149	298	2907	45	91	228	457	4398
100000	23	46	115	230	2276	29	59	149	298	2951	45	91	229	458	4499
Infinite	23	46	114	230	2301	29	59	149	299	2994	45	91	229	458	4603

This table is adapted from the approximation to the hypergeometric distribution provided by Roe and Cannon (1982, p. 30).