From the Field: Efficacy of detecting Chronic Wasting Disease via sampling hunter-killed white-tailed deer

Duane R. Diefenbach, Christopher S. Rosenberry, and Robert C. Boyd

Abstract Surveillance programs for Chronic Wasting Disease (CWD) in free-ranging cervids often use a standard of being able to detect 1% prevalence when determining minimum sample sizes. However, 1% prevalence may represent >10,000 infected animals in a population of 1 million, and most wildlife managers would prefer to detect the presence of CWD when far fewer infected animals exist. We wanted to detect the presence of CWD in white-tailed deer (*Odocoileus virginianus*) in Pennsylvania when the disease was present in only 1 of 21 wildlife management units (WMUs) statewide. We used computer simulation to estimate the probability of detecting CWD based on a sampling design to detect the presence of CWD at 0.1% and 1.0% prevalence (23–76 and 225–762 infected deer, respectively) using tissue samples collected from hunter-killed deer. The probability of detection at 0.1% prevalence was <30% with sample sizes of ≤6,000 deer, and the probability of detection at 1.0% prevalence was 46–72% with statewide sample sizes of 2,000–6,000 deer. We believe that testing of hunter-killed deer is an essential part of any surveillance program for CWD, but our results demonstrated the importance of a multifaceted surveillance approach for CWD detection rather than sole reliance on testing hunter-killed deer.

Key words Chronic Wasting Disease, *Odocoileus virginianus*, Pennsylvania, prevalence, probability of detection, sample size, sampling design, white-tailed deer

Chronic Wasting Disease (CWD) is a contagious, fatal disease affecting mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), and elk (*Cervus elaphus*). The disease is endemic to parts of Colorado and Wyoming, where it has been present for at least 30 years in populations of free-ranging cervids (Miller et al. 2000). No cure exists (Williams et al. 2002), and management actions after the disease has been detected usually involve drastic cervid population reductions (Bartelt et al. 2003).

Recent discoveries of CWD in free-ranging cervids in Wisconsin, New Mexico, Utah, and Illinois have heightened awareness of this disease. All, or nearly all, states in the continental United States have implemented some type of surveillance sampling to assess the probability that CWD exists there. However, current tests for CWD severely limit the ability to test large numbers of free-ranging cervids. Immunohistochemistry (IHC), the gold-standard test for detecting CWD, is a destructive test requiring death of the animal. Tonsillar biopsy IHC can detect CWD in live deer but requires capture and anesthesia of each animal and has <100% sensitivity and specificity (Wolfe et al. 2002).

Chronic Wasting Disease can be detected via samples from hunter-killed cervids (Conner et al. 2000, Bartelt et al. 2003). This method is advantageous because 1) the animal is already dead, 2) much of the cost of collecting the deer is borne by the hunter, and 3) the sample can be acquired read-

ily from a geographically large area. However, problems with this type of sampling are 1) if only a small percentage of the deer population is infected with CWD (i.e., low prevalence), the probability of a hunter-killed deer being infected is small, and 2) hunters likely do not harvest deer in such a manner that hunter-killed deer can be treated as a random sample from the population.

Surveillance programs of hunter-killed deer often base sample sizes on standard disease detection protocols (Samuel et al. 2003). Those protocols include a desired level of confidence (e.g., 95% or 99%) of detecting the disease at a given prevalence (e.g., 1%). These protocols result in the "95-1" or "99-1" rules for deciding on number of deer to sample. These rules typically are implemented in such a way that 300 or 500 animals are tested, and none are found to be infected, to be assured that the prevalence is <1.0% with 95% and 99% confidence, respectively. Although based on statistical methods, the 95-1 and 99-1 rules do not directly address the issue of number of infected animals that are present in the population. That is, if the population consists of 1,000 deer, 368 randomly selected animals must test negative to be 99% confident that prevalence is <1.0%, whereas only an additional 90 animals (n = 458) need to be tested if the population size is 100,000 animals. However, 1.0% prevalence at N = 1,000 results in 10 infected deer, whereas at 1.0% prevalence at N = 100,000 results in 1,000 infected deer. Like other agencies that have collected an initial sample of 300 or 500 deer to be tested for CWD, Pennsylvania began a CWD surveillance program in 2002 by testing 487 hunter-killed and 86 culled and road-killed white-tailed deer, and all 61 hunter-killed elk. In Pennsylvania, where the adult (≥1 year of age) white-tailed deer population was estimated to be approximately 1 million before the hunting season, this sample had a ≥99% chance of detecting ≥1 deer infected with CWD if prevalence was 1.0% and if all deer had an equal chance of being tested for CWD. Although this sample was statistically sufficient in number, the biological interpretation of the results (i.e., a 99% chance of detecting CWD if there were ≥10,000 deer infected with CWD) is less encouraging.

CWD represents a significant wildlife health concern in Pennsylvania because the state has >700 white-tailed deer and elk farms with >22,000 captive cervids (Pennsylvania Game Commission [PGC], unpublished data), has received live cervids and carcasses of hunter-killed cervids from CWD-affected regions, and supports high-density deer populations (26–29 deer/2.6 km^2 after the hunting season) across much of the state. Consequently, Pennsylvania meets most of the exposure risk factors identified by Samuel et al. (2003) as well as 2 factors that increase the risk of disease spread once present (high deer densities and feeding of free-ranging deer). If CWD were found in Pennsylvania, the economic impact would be significant because most of the >$900 million of hunting-related expenditures (United States Department of the Interior, Fish and Wildlife Service, and United States Department of Commerce, Census Bureau 2002) were related to deer hunting. To establish protocols for detecting the presence of CWD and how to respond if it is detected, an interagency task force (PGC, Department of Agriculture, Department of Health, Department of Environmental Protection, Governor's Policy Office, Emergency Management Agency, and the United States Department of Agriculture) was organized to prepare a CWD Response Plan for Pennsylvania.

CWD surveillance in Pennsylvania includes a 3-tiered monitoring approach. First, deer that exhibit clinical symptoms of the disease are collected and tested. This has been recommended as a useful approach for detecting presence of CWD (Miller et al. 2000, Samuel et al. 2003), but when the disease is not detected, the approach provides no statistical confidence on the probability that the disease is below a given prevalence. Second, Pennsylvania has begun collecting a sample of hunter-killed deer as well as all hunter-killed elk (<100 animals) each year to test for CWD. Third, the Pennsylvania Department of Agriculture has implemented a voluntary CWD monitoring program for captive cervids.

The purpose of this report is to present an estimate of the probability of detecting CWD presence by sampling hunter-killed deer and the associated cost of testing for CWD. We estimated these probabilities and costs by simulating likely sampling scenarios in Pennsylvania and compared these results to sampling recommendations obtained by applying the 99-1 rule.

Methods

We conducted computer simulations programmed in SAS (SAS Institute, Cary, N.C., USA) to estimate the probability of detecting CWD from a sample of hunter-killed deer. In the simulated sam-

Table 1. Area (km^2) and adult (≥1.5 yr old) white-tailed deer estimated population size for each wildlife management unit (WMU), as well as number of Chronic Wasting Disease-infected deer at 1.0% and 0.1% prevalence and the number of deer sampled (*n*) to be assured with 99% confidence that the prevalence is less than the specified level, Pennsylvania 2003.

WMU[a]	Area (km^2)	$\hat{N}$[b]	1.0% prevalence		0.1% prevalence	
			Infected	*n*	Infected	*n*
1A	4,782	40,382	404	456	40	4,350
1B	5,484	48,196	482	456	48	4,390
2A	4,690	47,877	479	456	48	4,388
2B	3,530	26,069	261	454	26	4,219
2C	8,028	76,211	762	457	76	4,467
2D	6,440	65,663	657	457	66	4,445
2E	3,267	33,314	333	455	33	4,299
2F	6,244	57,041	570	456	57	4,422
2G	10,655	52,450	525	456	52	4,407
3A	3,906	39,760	398	456	40	4,346
3B	5,830	53,297	533	456	53	4,410
3C	5,589	56,951	570	456	57	4,422
3D	5,654	51,687	517	456	52	4,404
4A	4,496	41,111	411	456	41	4,354
4B	4,112	41,942	419	456	42	4,359
4C	4,693	47,848	478	456	48	4,388
4D	7,112	67,509	675	457	68	4,449
4E	4,495	34,769	348	455	35	4,311
5A	3,369	22,518	225	454	23	4,163
5B	7,168	47,900	479	456	48	4,389
5C	5,620	37,531	375	455	38	4,332
Total	115,164	990,026	9,900	458	990	4,603

[a] WMU 5D, encompassing the city of Philadelphia, is excluded because population size is not estimated.

[b] Estimated minimum population size prior to the 2003 fall hunting season (Pennsylvania Game Commission, unpublished data).

pling design, we collected hunter-killed deer from primary sampling units defined as wildlife management units (WMUs) (Table 1). In Pennsylvania there are 22 WMUs organized according to areas with similar habitat characteristics (e.g., % forest cover), land-use characteristics (i.e., human development), and deer population characteristics (e.g., reproductive rates, harvest/km^2, etc.). Deer population estimates are available for all WMUs except the one that primarily encompasses the city of Philadelphia.

We made the following assumptions in our simulations: 1) deer were tested for CWD via a technique that has 100% specificity and 100% sensitivity, and whether a deer carcass was acceptable for testing (e.g., age of carcass, carcass condition, etc.) was unrelated to whether it was infected with CWD, 2) every adult deer (≥1.5 years old) within a sampling unit had the same probability of being tested, although we relaxed this assumption somewhat by treating prevalence as a random variable (explained in more detail hereafter), 3) total sample of deer tested for CWD was allocated among WMUs proportional to the estimated population size, and 4) only one WMU contained deer positive for CWD, but a sample of deer was obtained from every WMU.

Also, to attempt to incorporate some of the inherent variability in sampling hunter-killed deer, we treated deer-abundance estimates for each WMU as having precision of CV = 5% and modeled these estimates as a random variable with a Gamma (α,β) distribution, where $\beta = (CV/100) \times \hat{N}$ and $\alpha = \hat{N}/\beta$. We incorporated this variability into our simulations because the number of deer in each WMU was estimated (i.e., not known with certainty) and any error in these estimates would affect how sampling effort was allocated among WMUs. We assumed a precision of CV = 5% because the Pennsylvania Game Commission (PGC) estimates population size using an accounting-type model (Roseberry and Wolf 1991) and resulting population estimates do not have associated measures of statistical precision. The prevalence of CWD in the infected WMU was modeled as a random variable with a Beta(*a,b*) distribution, in which (*a,b*) was (1,99) or (0.1, 99.9) for 1.0% and 0.1% prevalence, respectively. We incorporated variability in the prevalence of CWD to relax the assumption that every animal in the population had the same probability of being sampled for CWD. For example, deer hunters are less likely to harvest deer >1 km from a road open to public vehicular travel (D. R. Diefenbach, unpublished data), and not all landowners permit hunting. Thus, if infected deer are located far from a road or on properties closed to hunting, their probability of being sampled is lower.

In the simulations we investigated 10 different scenarios: 5 sample sizes ranging from 2,000–6,000 deer, allocated among 21 WMUs, and for each sample size 2 rates of prevalence of CWD in the population (1.0% and 0.1%). For each scenario we conducted 10,000 simulations. A simulation consisted of the following steps:

1. The population size for each WMU was estimated [a random variable distributed as Gamma(α,β)], and the statewide sample size of deer tested for CWD was allocated among

WMUs proportional to estimated deer population size.
2. A WMU was randomly chosen to contain infected animals.
3. The number of infected animals was calculated as true population size multiplied by prevalence (random variable distributed as Beta(1,99) or Beta(0.1,99.9), of which ≥1 deer had to be infected.
4. A deer selected to be tested for CWD (sample size determined in step 1) was classified as infected if a uniform random variable was less than the prevalence (determined in step 3).
5. The number of infected deer detected was tallied.

For each scenario, we created a cumulative distribution of the number of deer infected with CWD that were detected. The proportion of 10,000 simulations in which ≥1 infected deer was detected was an estimate of the probability of detecting CWD. Likewise, the complement was an estimate of the probability that the disease would fail to be detected.

Results

The probability of detecting CWD when prevalence was 0.1% was 0.15–0.29 (Table 2), and ≥89% of simulations that detected ≥1 infected individuals detected ≤4 infected deer (Figure 1). The reason for this low probability of detecting CWD was that there were few infected deer. Sixty-two percent of simulations had <50 infected deer available to be detected in the state, and 86% of the simulations had <250 infected deer.

Table 2. Probability of detecting zero and ≥1 adult (≥1.5 yr old) white-tailed deer infected with Chronic Wasting Disease (CWD) for 5 sample sizes of tested deer (allocated among WMUs in proportion to deer abundance) and 2 prevalence values. Simulations (n = 10,000) were conducted for testing deer from each of 21 WMUs in Pennsylvania containing 990,026 deer.

		Probability	
Prevalence (%)	No. deer tested	CWD not detected	≥1 CWD infected deer detected
0.1%	2,000	0.85	0.15
	3,000	0.81	0.19
	4,000	0.77	0.23
	5,000	0.74	0.26
	6,000	0.71	0.29
1.0%	2,000	0.54	0.46
	3,000	0.43	0.57
	4,000	0.38	0.62
	5,000	0.31	0.69
	6,000	0.28	0.72

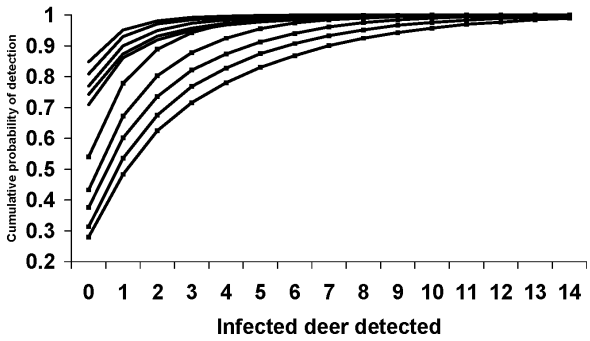

Figure 1. Cumulative probability distribution of the number of adult deer (≥1.5 years old) infected with Chronic Wasting Disease (CWD) that were detected when mean prevalence was 0.1% (lines without markers) or 1.0% (lines with markers). Curves, from top to bottom (for each prevalence value), are for Pennsylvania statewide sample sizes of 2,000, 3,000, 4,000, 5,000, and 6,000 adult deer tested for CWD.

The probability of detection was 0.46–0.72 when prevalence was 1.0% (Table 2), and ≥89% of simulations that detected ≥1 infected individuals detected ≤8 infected deer (Figure 1). Ten percent of simulated populations had <50 infected deer available for detection, 48% had <250 infected deer, and 67% had <500 infected deer.

The estimated cost of collecting and transporting heads from 2,100 hunter-killed deer and testing for CWD is approximately $56 per sample in Pennsylvania (R. C. Boyd, unpublished data). Using this estimated figure, 2,000–6,000 samples would cost $112,000–$336,000. However, to have a >50% probability of detecting CWD when prevalence is ≤0.1%, statewide >25,000 deer would have to be tested at a cost of >$1.4 million.

Discussion

Despite the low probability of detecting CWD with sample sizes investigated in these simulations, our results probably represent best-case scenarios. Violation of any one of the assumptions would likely lead to lower detection rates. For example, if the test used to detect CWD has <100% specificity, our simulations overestimated detection probabilities. In addition, the probability of a deer being killed by a hunter or having a tissue sample collected for testing likely differs among deer, which could further reduce the probability of detecting CWD.

At least one deer must have a >0 probability of being selected for testing if CWD is to be detected. In our computer simulations, once the number of deer infected with CWD was determined, every deer had the same probability of being tested for CWD. However, to partially relax the assumption of homogeneity in probability of selection, we specified the prevalence of CWD to be a random variable. That is, in some simulations the number of infected deer was less than the specified prevalence (and vice versa), which would simulate situations in which infected deer were less (more) likely to be sampled (e.g., infected deer might inhabit land closed to hunting and thus be less likely to be killed by hunters). However, we always required that ≥1 deer be available for testing.

We believe the probability of deer being sampled for testing is heterogeneous, as is the probability of a deer being harvested by a hunter. The probability of a deer being killed by a hunter likely depends on such factors as location (e.g., whether the land is open to hunting), hunting season (e.g., harvest rates during the archery season are lower than during the rifle season), age and sex of the animal (e.g., mature bucks have lower probability of being harvested than females or yearling males), hunter preferences, and possibly many other factors. Unfortunately, data are not available to empirically estimate how the probability of harvest differs among deer in Pennsylvania. Likewise, the probability of being selected for CWD testing is likely heterogeneous among deer; adult males with large antlers are likely to have been taken to a taxidermist before agency personnel can collect tissue samples, and antlerless deer are more likely to be killed by a gunshot to the head. Both of these situations compromise the quality of brain tissue used to assess the presence of CWD. Consequently, we believe that specifying the number of infected deer in a WMU as a random variable was a reasonable approach to incorporating heterogeneity in sampling probabilities among deer.

We conducted simulations of the probability of detecting the presence of CWD because the 99-1 approach suggested a deceptively small sample size if one does not consider the implications of 1% prevalence in a population of nearly 1 million animals. Moreover, the statistical confidence of detecting this prevalence was likely <99% because of violation of assumptions regarding random probability of harvest or sampling. In Pennsylvania wildlife managers are interested in detecting CWD well before nearly 10,000 deer are infected (Table 1). Preferably, the disease would be detected when <100 deer are infected in a given WMU, but this objective may be unrealistic in Pennsylvania using a surveillance program based solely on hunter-killed deer. Our simulations indicated that sample sizes for testing hunter-killed deer that provided >70% chance of detecting CWD when the disease was prevalent at a rate <1.0% would be costly and logistically difficult to collect.

One method of increasing sample sizes is to pool data over several years of sampling effort. Thus, if 2,000 deer were sampled per year and data from 2003 and 2004 were pooled, the sample could be considered equivalent to 4,000 tested deer. However, the resulting confidence that prevalence of CWD is <1% based on n=4,000 is valid only if CWD were not introduced during the sampling period (Samuel et al. 2003). We would interpret such estimates from pooled data with caution, but based on Table 2 would estimate a 62% chance of detecting CWD in Pennsylvania in 2003 (at ≥1.0% prevalence in a given WMU) and only 46% chance of detecting CWD in 2004.

Another approach to try to improve the efficiency of a CWD sampling program would be to define biologically appropriate sampling units. The PGC established spatially explicit WMUs with similar deer population characteristics (e.g., reproductive rates), physiography, and human land use (http://www.pgc.state.pa.us/land/wmu/index.asp). Unlike some cervid populations in western states, we have no evidence to delineate demographically distinct subpopulations of deer (e.g., subpopulations with population-specific areas where they spend the winter or summer). Hence, WMUs in Pennsylvania are relatively homogeneous areas that are as small as possible but still allow agency personnel to collect sufficient data to estimate population parameters (e.g., population size, reproductive rates, hunter harvest, etc.). We believe that WMUs, as defined for Pennsylvania, would be most appropriate for monitoring deer populations in the eastern United States.

Management implications

We considered the testing of hunter-killed deer to be an important strategy for CWD surveillance in Pennsylvania. This testing was designed to 1) provide an additional method of detecting CWD, 2) train and test personnel and infrastructure for col-

lecting carcasses, extracting sample tissue, and testing samples, 3) support baseline data collection on whether CWD is present in Pennsylvania, and 4) inform and educate hunters and citizens about CWD. However, given the cost of obtaining sample sizes that provided >90% chance of detecting CWD at <1.0% prevalence at the WMU primary sampling unit, we do not recommend relying solely on sampling hunter-killed deer as a surveillance strategy.

In Pennsylvania we supplemented testing of hunter-killed deer with testing of deer that exhibit clinical symptoms consistent with CWD, often called targeted surveillance. Although targeted surveillance provides no statistical measure of prevalence, or confidence that disease prevalence is less than a specified level, it is believed to aid in detection of the disease at low prevalence (Miller et al. 2000, Samuel et al. 2003). Also, we believe that captive-cervid surveillance is critical to the early detection of CWD because captive-cervid facilities are considered a risk factor that increases the chance of introducing CWD as well as contributing to its spread (Samuel et al. 2003).

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