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# Chronic Wasting Disease Surveillance in Montana 1998-2011 A Summary of Surveillance Efforts

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## Background

Chronic Wasting Disease (CWD) is a fatal neurologic disease of elk, deer and moose for which there is no known cure. CWD belongs to a group of diseases called transmissible spongiform encephalopathies (TSE). TSEs are unique in that the causative agent is thought to be an aberrantly shaped protein (prion) that has the ability to transform cellular proteins into disease causing forms (Prusiner 1998). The prion associated with CWD ($PrP^{cwd}$) produces lesions in the gray matter of the central nervous system resulting in a spongiform change in animal showing clinical symptoms for CWD (Williams 2005). Lesions can be detected in many regions of the brain, but are prominent in the olfactory cortex, nuclei of the medulla oblongata (primarily the dorsal vagal nucleus) and the diencephalon (Williams and Young 1993, Spraker et al. 2002, Williams 2005). Although considered a neurologic disease, $PrP^{cwd}$ has been detected in tonsil and lymph tissue of elk, deer (Sigurdson et al. 1999, O'Rourke et al. 2003, Spraker et al. 2004, Williams 2005, Race et al. 2007) and moose (Baeten et al. 2007), the intestinal tract, urinary bladder, blood, and saliva of white-tailed deer (Haley et al. 2011), saliva and blood of mule deer (Mathiason et al. 2006), urine and feces of white-tailed deer (Haley et al. 2009, Haley et al. 2011), feces of mule deer (Tamguney et al. 2009) and elk (Pulford et al. 2012), muscle of mule deer (Angers et al. 2006) and white-tailed deer (Duas et al. 2011), fat from mule deer (Race et al. 2009), and antler velvet (Angers et al. 2009). The presence of $PrP^{cwd}$ in the various tissues can vary, dependent on the stage of the disease, the species affected and even among individuals of the same species. CWD is only known to infect elk, mule deer, white-tailed deer and moose.

Symptoms of CWD vary as well. Descriptions of clinical signs of CWD are based on observations of captive elk and deer (Williams and Young 1982, Williams 2005) and are less well known in free-ranging wildlife. Early symptoms are subtle behavioral changes and may not be discernible in free-ranging animals. CWD is generally characterized by progressive weight loss and behavioral changes. Symptoms may also include excessive salivation, ataxia, head tremors, grinding of teeth, excessive thirst, excessive urination, changes in posture, lack of awareness, fixed stare, changes in interactions with herd mates, lowered head, repetitive walking, hyper excitability, regurgitation, and asphyxiation pneumonia. Hair can appear rough and dry,

likely due to poor body condition. The above symptoms vary among individuals and become more evident in terminal stages. Deer with subclinical or early clinical CWD are more susceptible to handling mortality (Miller and Williams 2003). Aspiration pneumonia can occur, even in the early stages of the disease, so any case of pneumonia should be considered suspect for CWD.

Environmental contamination with $PrP^{cwd}$ in areas occupied by CWD positive animals may occur and could influence the persistence of the disease in cervid populations (Gough and Madison 2010). Several studies have demonstrated the ability of prions to bind to soils (Johnson et al. 2006, Johnson et al. 2007, Seidel et al. 2007, and Saunders et al. 2011), although prions have yet to be found in naturally contaminated soils (Gough and Madison 2010). Low levels of $PrP^{cwd}$ were detected in environmental water samples, and samples from a water treatment facility within a CWD endemic area of Colorado. However, $PrP^{cwd}$ levels were not considered to be an infective dose based on bioassays (Nichols et al. 2009). The potential of vegetation to contain and possibly accumulate $PrP^{cwd}$ during uptake of nutrients and water from contaminated environments is currently unknown.

The route of transmission for CWD is not well understood, but evidence suggests both direct (Miller and Williams 2003) and indirect transmission (Williams and Young 1982, Miller et al. 1998, Miller et al. 2004, Mathiason et al 2009) may be possible. $PrP^{cwd}$ presence in bodily fluids could result in direct or horizontal transmission. The detection of $PrP^{cwd}$ in urine and feces of CWD infected cervids (Bosque et al. 2002, Haley et al. 2009, Haley et al. 2011, Pulford et al. 2012) and the ability to produce CWD in naïve animals after exposure to infected urine or feces supports the possibility of indirect transmission through environmental contamination. Carcasses from animals infected with $PrP^{cwd}$ can also lead to environmental contamination and pose a source for $PrP^{cwd}$ for other cervids utilizing the area (Miller et al. 2004).

Establishing incubation periods for CWD is problematic due to difficulty in determining onset of the disease. Environmental conditions and stress can also influence survival of CWD infected individuals, particularly in free-ranging wildlife. In captive animals, incubation periods for CWD can be prolonged with death typically occurring within months to a year after observing clinical signs (Williams 2005). Sudden or short-duration deaths are rare but have been observed (Miller and Williams 2004). $PrP^{cwd}$ has been observed in the alimentary tract of a mule deer 42 days following experimental exposure (Sigurdson et al. 1999). Average incubation periods range from two to four years, therefore yearlings typically do not demonstrate clinical disease (Williams 2005). The detection of $PrP^{cwd}$ in feces of subclinical animals experimentally infected with CWD suggests the ability to shed infectious prions before symptoms of CWD are apparent (Tamguney et al. 2009).

Currently there is no known cure for CWD in cervids, and complete genetic resistance has not been documented (O'Rourke et al. 1999, O'Rourke et al. 2004, Johnson et. al 2006, Johnson et. al 2011). However, polymorphisms in the prion producing (PRNP) gene tend to be overrepresented in CWD positive animals, suggesting variable susceptibility (O'Rourke et al. 1999, O'Rourke et al. 2004, Wilson et al, 2009, White et al. 2010, Johnson et. al 2011). Although genetic resistance to CWD has yet to be documented, incubation period does seem to be influenced by PRNP polymorphism. Incubation times for deer heterozygous for the PRNP

alleles were longer than those of homozygous PRNP deer (Johnson et al. 2011). Whether these observed variations in susceptibility and incubation times will influence CWD's impact on populations in affected areas is not known.

The ultimate effect CWD may have on wildlife populations is poorly understood and is likely a function of many factors (Miller and Conner 2005). Early models predicted possible decimation of populations as a result of CWD (Goss and Miller 2001). More recent models suggest a wide variety of possible effects on deer population dynamics that are dependent on several variables including: transmission route (direct or indirect), persistence of prions in the environment, and whether CWD transmission is density dependent, frequency dependant, or some combination of both. The varying prevalence and incubation times observed in PRNP heterozygous and homozygous cervids has not been addressed in population models. If or how genetic structure will influence CWD's effect on populations is not well understood. Likewise, how CWD may influence the genetic composition of populations in long established endemic areas is unknown. Although models use available information in estimating effects on populations, often educated guesses are made regarding many of the possible variables affecting CWD transmission and prevalence of the disease. The most plausible models suggested populations would persist, but at lower numbers (Almberg 2011).

CWD prevalence has been observed to increase over time in affected populations of Colorado (Miller and Conner 2005), Wyoming (Almberg et al. 2011) and Wisconsin (Heisey 2010). CWD prevalence in mule deer populations has been estimated to exceed 30% in endemic regions of Wyoming (Almberg et al. 2011) and 20% in the Table Mesa area of Colorado (Miller et al. 2008, Dulberger et al. 2010). Estimates of CWD prevalence in Wisconsin white-tailed deer harvested by hunters in the core CWD area were approximately 7% and 17% for adult females (Wisconsin Department of Natural Resources 2012a) and males (Wisconsin Department of Natural Resources 2012b), respectively. A prevalence exceeding 12% was observed in elk from Rocky Mountain National Park, CO (Monello 2012). Population declines have been observed in some areas with high CWD prevalence (Miller et al. 2008) and increased predation rates of CWD infected deer have been reported (Miller et. al. 2008, Krumm 2010). The influence predation may have on populations with high levels of CWD is not entirely clear (additive versus compensatory mortality), but at least one model suggests that selective predation of CWD infected individuals may have a dampening effect on CWD prevalence (Wild et al. 2011). There are many unanswered questions regarding the potential effects of this disease on wildlife populations, but current literature suggests that as prevalence increases population declines may be observed.

**CWD Surveillance in Montana**

Montana Fish, Wildlife and Parks initially started conducting surveillance for CWD in 1996, collecting 63 samples from hunter-harvested elk in the Greater Yellowstone area, although there was not an established surveillance plan. Dedicated funding was not allocated to CWD surveillance until 1998 when the governor issued a directive for MFWP and DoL to work together on surveillance and control of CWD. At that time MFWP allocated approximately $23,000 for CWD surveillance activities. Additional funding was supplied through cooperative agreements with USDA-APHIS on a year-by-year basis until 2004. MFWP also took advantage

of research projects offering to conduct testing in exchange for samples. Non-MFWP funding varied by year, was provided on a limited basis, and constituted a small portion of the overall cost of CWD surveillance until 2004. In 2002, the national *Plan to Assist States, Federal Agencies and Tribes in Managing Chronic Wasting Disease in Captive and Free Ranging Cervids* was drafted and, as a result, USDA-APHIS paid for testing costs for CWD in 2002 and 2003. In 2004 USDA coverage of testing costs was eliminated and a grant system was implemented which provided funding to states for CWD surveillance in free-ranging wildlife through an application process. MFWP received $89,000 from USDA-APHIS Veterinary Services for CWD surveillance for the 2004 survey season. That amount increased to $90,000 for 2005 and 2006, but was reduced to $75,000 in 2007 and again to $70,000 for 2010 and 2011. Federal funding for state CWD surveillance and management was eliminated in 2012. The initial goal of CWD surveillance was to determine whether CWD was present in Montana's wild cervid populations and, if present, detect the disease early. Although changes were made to surveillance strategies over the years, the goal has basically remained the same, early detection.

Samuel et al. (2003) recommended a multi-tiered approach utilizing hunter-harvested samples, targeted testing of symptomatic animals, and testing samples from road kills or natural mortalities (as available) when developing CWD surveillance strategies. When identifying priority areas for surveillance five criteria were to be considered: 1) proximity to CWD-positive wildlife, 2) proximity to land on which TSE-positive animals have lived (both wild and farmed), 3) presence of farmed or captive elk or deer, 4) translocation efforts from CWD-affected regions, and 5) the potential movement of hunter-killed elk or deer from CWD infected areas. Sample size goals were based on a statistical confidence of detecting a single case of CWD at an estimated prevalence and population size, and adapted from an approximation of the hypergeometric distribution provided by Roe and Cannon (1982) (Samuel et al. 2003). MFWP utilized these suggestions and modified surveillance strategies as new information became available to increase the probability of detecting CWD in Montana's free-ranging wildlife.

MFWP relied on the reported distribution of CWD in wildlife populations of adjacent states and provinces, animal movement information for wildlife populations within Montana obtained from research projects and expert opinion, and literature published on CWD to define high risk areas where CWD would most likely be detected if it were present in the state (Figure 1). Surveillance primarily focused on these "high risk" areas, applying the tiered approach recommended by Samuel et al. (2003). Hunter-harvested and road killed animals were utilized to obtain large sample sizes in "high risk" areas and testing of symptomatic animals was used for state-wide surveillance. Sample size goals in high risk areas were based on achieving a large enough sample to detect a single CWD case from a large population at an estimated prevalence of 1%, and at a predefined level of confidence. Hunter-harvested animals were used because they were relatively convenient to obtain and provided a source for large numbers of samples, although sample size goals for survey populations often were not met (Anderson et. al. 2010, Anderson et. al. 2011, Anderson et. al. 2012). Below is a summary of the different CWD surveillance strategies MFWP utilized from 1998 through 2011. Just as the sample collection and locations changed over time, so did the primary tissues tested. The tissues tested and the tests used were based on the available information and the development and approval of new tests. The primary tests used consisted of IHC on fixed tissue and ELISA fresh tissue (Table 1).

Testing costs for IHC and ELISA performed on tissues remained fairly consistent, being $25 and $17 per sample, respectively.

## Montana CWD Survey Methods

### 1998-1999

The goal of surveillance in 1998 and 1999 was to conduct broad geographical surveillance across the state in an effort to get baseline information on CWD presence or absence. During the 1998 and 1999 hunting seasons, elk and deer heads were collected from hunters on a voluntary basis at game check stations and designated drop points across Montana. Kits containing information about CWD, the location of game check stations and drop points, and collection protocols were mailed to deer and elk permit holders. Technicians were employed to work check stations and collect heads for testing. Symptomatic animals from across the state were tested for CWD.

### 2000-2001

In 2000 surveillance efforts shifted from statewide collections to collection of samples in areas considered to be "high risk" for movement of CWD into the state. In January 2000, deer and elk from the area surrounding the Kessler Game Farm near Philipsburg, MT were lethally removed by ground based sharpshooters and through aerial gunning from a helicopter, following detection of CWD in elk from the game farm in 1999. MFWP also participated in the removal and testing of mule deer from the Sunlight Game Farm near Hardin, MT. The Sunlight Game Farm was going out of business and wanted to remove its fences. It also shared a fence line with the Elk Valley Game Farm, which had previously received elk from Kessler's Game Farm. The Elk Valley Game Farm was under quarantine and ultimately depopulated in June of 2000. MFWP assisted in the depopulation and testing of elk from Elk Valley as well. Surveillance during the fall hunting season focused on the area surrounding the Kessler Game Farm near Philipsburg and the southeastern border with Wyoming. These two areas were selected because of concern over the potential transmission of CWD from infected game farm elk and the potential movement of CWD infected deer into Montana from northeastern Wyoming. Heads from hunter-harvested deer and elk were collected at check stations and drop points within the Philipsburg and southeastern border areas. Surveillance in 2001 again focused on the Philipsburg area, but efforts in eastern Montana shifted to the northeastern border with Saskatchewan rather than the southeastern border with Wyoming, a result of the detection of CWD in wild deer near the Saskatchewan-Montana border. Statewide surveillance consisted of testing symptomatic animals reported to MFWP.

### 2002 – 2005

USDA-APHIS agreed to cover laboratory testing costs in 2002 and 2003, and federal funding of the national "*Plan to Assist States, Federal Agencies and Tribes in Managing Chronic Wasting Disease in Captive and Free Ranging Cervids"* in 2004 allowed for increased surveillance for CWD. As a result, surveillance was conducted simultaneously in "high risk" areas near Montana's northeastern border, southeastern border, southern border with

Yellowstone National Park, and the Philipsburg area. The Philipsburg area was dropped from the "high risk" designation and collection of hunter-harvested and road-killed animals was discontinued in 2004 Voluntary participation by hunters remained the primary method of collecting elk and deer heads in the remaining areas. Collections occurred at drop barrels, game check stations and selected game processors. Cooperating game processors were paid $1 per head collected. Efforts were taken to improve the accuracy of information regarding harvest location, and databases were updated to conform to national standards. Targeted surveillance continued for all symptomatic elk and deer reported to the MFWP Wildlife Laboratory.

Sample size goals for the 2003 and 2004 surveys were 500 samples from FWP administrative regions 3, 5, 6 and 7, and 450 samples in FWP administrative region 2 (2003 only). These goals exceeded recommendations proposed by Samuel et al (2003) and considered sufficient to detect one CWD positive animal from a large populations assuming a 1% infection rate and a 99% confidence interval. Collection points were located throughout the survey area in an effort to achieve an even distribution of samples across the surveillance area in regions 2, 5, 6 and 7. In 2004 and 2005 sample size goals were reduced to 400 samples and surveillance concentrated on the "high risk" areas of regions 3, 5, 6, and 7. Goals were reduce to be more consistent with Samuel et al. (2003), and were believed to be more achievable, based on previous experience.

A research project assessing mule deer demographics and potential transmission of CWD through deer movements between Wyoming and Montana was initiated in the winter of 2004-2005 (see Carnes 2009). In conjunction with the study, surveillance goals were increased by 200 samples for the southern portion of the survey area in region 7. A cooperative agreement was also reached with the Charles M. Russell Wildlife Refuge (CMR) to collect an additional 200 samples from hunting districts containing refuge lands in the northeastern portion of Montana in 2004.

Game processors within regions 5, 6 and 7 were contacted to solicit cooperation starting in 2002. Samples in region 3 were collected primarily from check stations within the Madison Valley west of YNP and a game check station located north of Gardiner, Montana during the late elk hunt in the months of January and February. A cooperative agreement was reached with the National Park Service for payment of testing costs for samples from elk harvested during the Gardiner late hunt (which consists primarily of elk migrating from Yellowstone National Park) in 2004 and 2005. In an effort to improve location information associated with each sample, game processors and drop barrels were not used during the 2005 general hunting in region 7. Technicians obtained samples during game check station operations by collecting road kills, through assistance of taxidermists and contacts with hunters in the field.

**2006-2008**

Surveillance in 2006-2008 was conducted in a similar manner to the surveys conducted since 2003, with a few exceptions. Collection of samples from hunter-harvested animals was not conducted in region 3 due to decreases in federal funding and in attempts to focus efforts in eastern Montana, which was viewed as a higher priority area. Goals for regions 5, 6 and 7 remained at 400 samples from each region with the exception of region 6. Within this region the

goal was 400 samples in areas north and east of hunting districts containing CMR lands. MFWP entered into a cooperative agreement with the CMR to conduct surveillance in hunting districts containing CMR lands during 2006-2008. In this area, efforts were maximized to collect as many samples as possible given the remote locations and difficulty in accessing hunters. Within region 7 the goal was 400 samples with a minimum of 200 coming from the southern portion of the survey area along the border with Wyoming.

**2009-2011**

The discovery of a CWD positive moose near Jackson Hole, WY in 2008 raised concern that CWD might find its way into the elk feedgrounds in Wyoming. As a result, the southern portion of region 3 was again elevated to a high risk area. Due to the large area in Montana considered to be at high risk for movement of CWD into the state and limited available funding, the state was divided into two surveillance areas. The eastern half of the state, primarily regions 4, 6 and 7, comprised one area and the south central and southwestern portions of the state, primarily regions 3 and 5 comprised the other area. Surveillance activities alternated between the two areas, occurring in the central/southwest one year and the eastern regions the following year. Surveillance efforts focused on regions 3 and 5 in 2009.

Prior to 2009, elk, mule deer and white-tailed deer samples were pooled when determining sample size goals, in effect considering the three species one population. Starting in 2009, each species within a MFWP region was considered a unique population. Sample size goals for each population were based on recommendations within the USDA-APHIS cooperative agreement and based on statistical tables referenced by Samuel et al. (2003). Those goals consisted of collecting a large enough sample to be 95% confident that we would detect a single CWD positive animal assuming a 1% infection rate. However, in areas and for species where harvest was limited, the sampling goal was 25% of the prior hunting season's estimated harvest. Moose were tested on a state-wide level as available. The cooperative agreement with the CMR to conduct surveillance in hunting districts containing CMR lands was renewed in 2009 but discontinued in 2010 and 2011. The goal for the CMR sampling effort was to maximize sample sizes for white-tailed deer, mule deer and elk. Local game processors were offered a reimbursement of $5 for elk and deer heads collected within the surveillance area in an effort to increase the numbers of samples and the quality of data associated with the samples.

Table 1. Tissues collected, primary tests conducted, and primary laboratories used for CWD surveillance activities in Montana, 1998-2005. Primary labs used include National Veterinary Services Lab (NVSL) and Colorado Veterinary Diagnostic Lab, Colorado State University (CSU).

| Year | Tissues Collected | Primary Tissue Tested | Primary Lab Used | Primary Test |
|---|---|---|---|---|
| 1998 - 2001 | Brain, Tonsil, Retropharyngeal Lymph Nodes | Brain | NVSL | IHC |
| 2002 | Brain and Retropharyngeal Lymph Nodes | Retro's for deer Brain for elk | NVSL | IHC |
| 2003 - 2011 | Brain and Retropharyngeal Lymph Nodes | Retro's for elk and deer Retro's and brain for moose | CSU | ELISA |

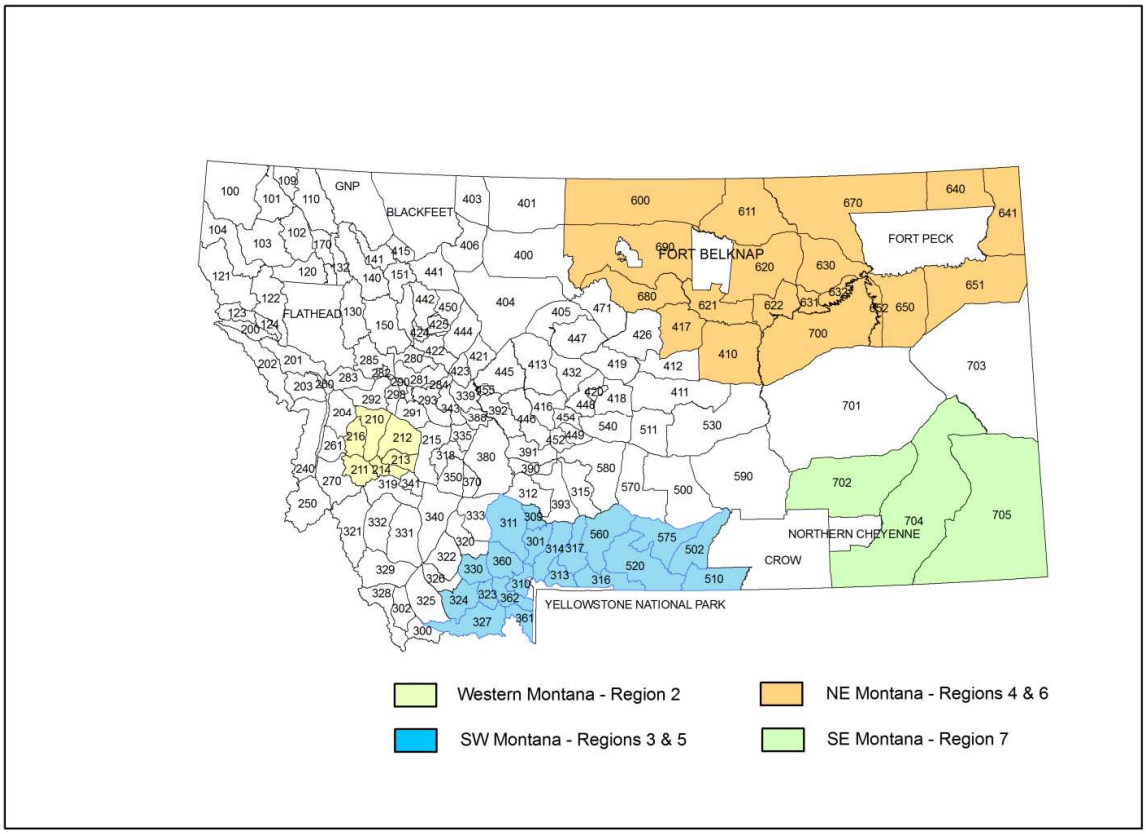



Figure 1. CWD surveillance areas for hunter-harvested and road-killed elk and deer collected from 1998-2011. Surveillance was conducted throughout Montana in 1998 and 1999. The majority of surveillance efforts from 2000 to present focused on the highlighted areas which were considered "high risk" for CWD based on proximity to known CWD cases in captive elk or free-ranging elk, deer and moose in other states and provinces. Survey efforts within each area (Western, NE, SW and SE Montana) varied by year and the goals established for the season.

**Montana CWD Survey Results**

From 1998 through the 2011 survey season, 17,269 samples from free-ranging elk, deer and moose were tested for the presence of $PrP^{cwd}$ as part of MFWP's annual CWD surveillance program. The number of samples tested varied by year and region (Table 2, Figure 2), and was dependent on the goals of the surveillance program as referenced above. Regions 5, 6 and 7 accounted for over 73% of the samples collected from 1998-2011, and sampling effort in these areas increase considerably after MFWP began focusing surveillance in SW, NE and SE Montana beginning in 2004 (Table 2). Elk, moose, mule deer and white-tailed deer comprised 20.4%, 0.7%, 55.4% and 23.5% of the samples tested, respectively (Table 3). The primary species tested varied by survey season (Table 3) and general area of the state (Figure 3). Hunter-harvested animals consistently comprised the majority of samples tested, accounting for 93.5% of all samples; however, increased emphasis was placed on collecting road-kills starting in 2004 (Table 4). Targeted surveillance of symptomatic animals remained a focus of state-wide surveillance, but overall numbers varied greatly by season (Table 4).

Table 2. CWD samples collected within each MFWP administrative region, by survey season.

| Season | 1 | 2 | 3 | 4 | 5 | 6 | 7 | **Total** |
|---|---|---|---|---|---|---|---|---|
| 1998 | | 26 | 27 | 124 | 162 | 4 | 101 | 444 |
| 1999 | 86 | 20 | 40 | 142 | 73 | 96 | 125 | **582** |
| 2000 | | 222 | 33 | 2 | 2 | 3 | | **262** |
| 2001 | 2 | 148 | 13 | 4 | 2 | 37 | 2 | **208** |
| 2002 | | 74 | 139 | 5 | 330 | 255 | 194 | **997** |
| 2003 | 4 | 254 | 435 | 9 | 363 | 586 | 409 | **2060** |
| 2004 | 3 | 4 | 417 | 79 | 428 | 743 | 750 | **2424** |
| 2005 | 1 | | 256 | 105 | 536 | 543 | 645 | **2086** |
| 2006 | 3 | 2 | 18 | 118 | 306 | 488 | 429 | **1364** |
| 2007 | | | 9 | 257 | 230 | 576 | 438 | **1510** |
| 2008 | 1 | | 11 | 184 | 271 | 1202 | 362 | **2031** |
| 2009 | 26 | 2 | 575 | 84 | 542 | 84 | 9 | **1322** |
| 2010 | 9 | 2 | 21 | 37 | 1 | 688 | 372 | **1130** |
| 2011 | 1 | | 546 | 3 | 297 | 1 | 1 | **849** |
| **Total** | **136** | **754** | **2,540** | **1,153** | **3,543** | **5,306** | **3,837** | **17,269** |

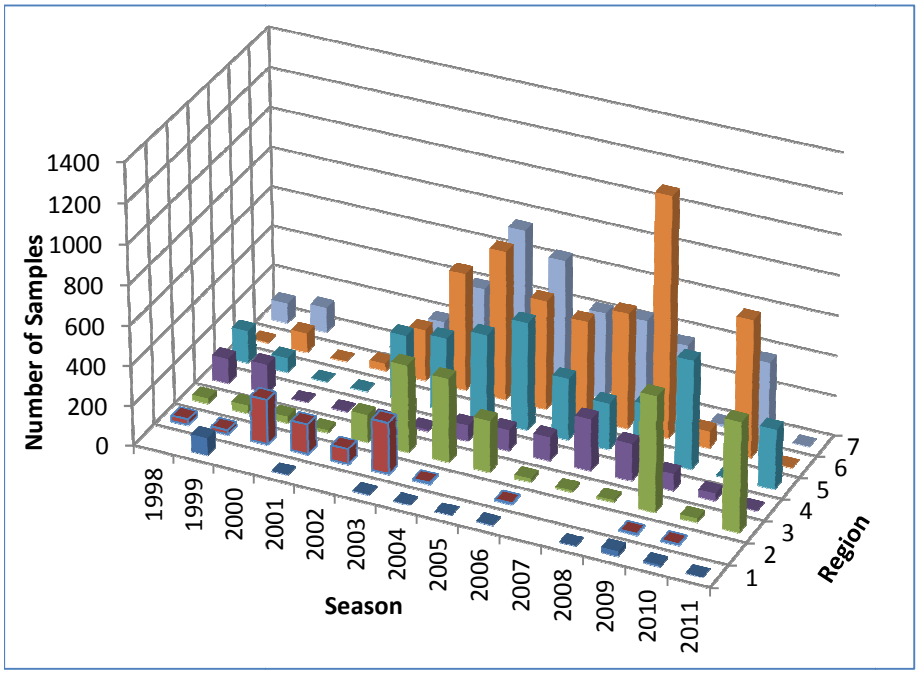



Figure 2. CWD samples from free-ranging elk, deer and moose collected within each region by survey season.

Table 3. CWD samples tested in Montana by season and species from 1998-2011.

| Season | Elk | Moose | Mule Deer | White-tailed Deer | **Total** |
|---|---|---|---|---|---|
| 1998 | 47 | 0 | 256 | 141 | **444** |
| 1999 | 46 | 0 | 336 | 200 | **582** |
| 2000 | 202 | 2 | 39 | 19 | **262** |
| 2001 | 112 | 0 | 69 | 27 | **208** |
| 2002 | 213 | 0 | 591 | 193 | **997** |
| 2003 | 714 | 1 | 880 | 465 | **2060** |
| 2004 | 612 | 0 | 1349 | 463 | **2424** |
| 2005 | 387 | 1 | 1254 | 444 | **2086** |
| 2006 | 226 | 3 | 900 | 235 | **1364** |
| 2007 | 178 | 1 | 1039 | 292 | **1510** |
| 2008 | 124 | 4 | 1298 | 605 | **2031** |
| 2009 | 368 | 74 | 563 | 317 | **1322** |
| 2010 | 68 | 20 | 677 | 365 | **1130** |
| 2011 | 222 | 14 | 317 | 296 | **849** |
| **Total** | **3,520** | **118** | **9,568** | **4,062** | **17,269** |

Table 4. Method of collection for CWD samples tested in Montana from 1998-2011.

| Season | Hunter | Road Kill | Target | **Total** |
|---|---|---|---|---|
| 1998 | 441 | 1 | 2 | **444** |
| 1999 | 575 | 3 | 4 | **582** |
| 2000 | 240 | 2 | 20 | **262** |
| 2001 | 189 | 1 | 18 | **208** |
| 2002 | 976 | 4 | 17 | **997** |
| 2003 | 2010 | 3 | 47 | **2060** |
| 2004 | 2333 | 68 | 22 | **2423** |
| 2005 | 1970 | 77 | 39 | **2086** |
| 2006 | 1166 | 114 | 84 | **1364** |
| 2007 | 1408 | 86 | 16 | **1510** |
| 2008 | 1806 | 219 | 6 | **2031** |
| 2009 | 1203 | 110 | 9 | **1322** |
| 2010 | 1053 | 68 | 9 | **1130** |
| 2011 | 768 | 70 | 11 | **849** |
| **Total** | **16,138** | **826** | **303** | **17,268** |

MFWP also collected (lethally removed) animals and tested samples under what were considered to be "special" circumstances (Table 5). These special collections occurred largely in conjunction with alternative livestock operations, and test results are not included in the surveillance summaries above. Under the special collection designation, free-ranging elk and deer were lethally removed from the area near the Philipsburg alternative livestock facility in 2000 following the finding of CWD positive domestic elk. MFWP also participated in the removal and testing of mule deer from the Sunlight Game Farm, depopulation and testing of elk from the Elk Valley captive herd, and depopulation and testing of fallow deer from a game farm near Bozeman. In all three incidences the owners chose to get out of the alternative livestock business. MFWP contributed personnel and, on occasion, testing costs to those efforts. In addition, elk that escaped from alternative livestock facilities were lethally removed and tested for CWD under the special collection designation. Heads from hunter-harvested elk and deer brought into Montana from states with CWD in free-ranging populations were also tested when the activity was reported and samples made available. Montana currently has restrictions on bringing cervid heads and body parts containing brain or spinal cord into the state from known CWD areas. Samples collected within the National Bison Range by the USFWS, who shared results with MFWP, were also considered special collections as the National Bison Range is enclosed by a fence. In total, 199 animals were tested under the special collection designation (Table 5) and not included in the above summaries of surveillance data.

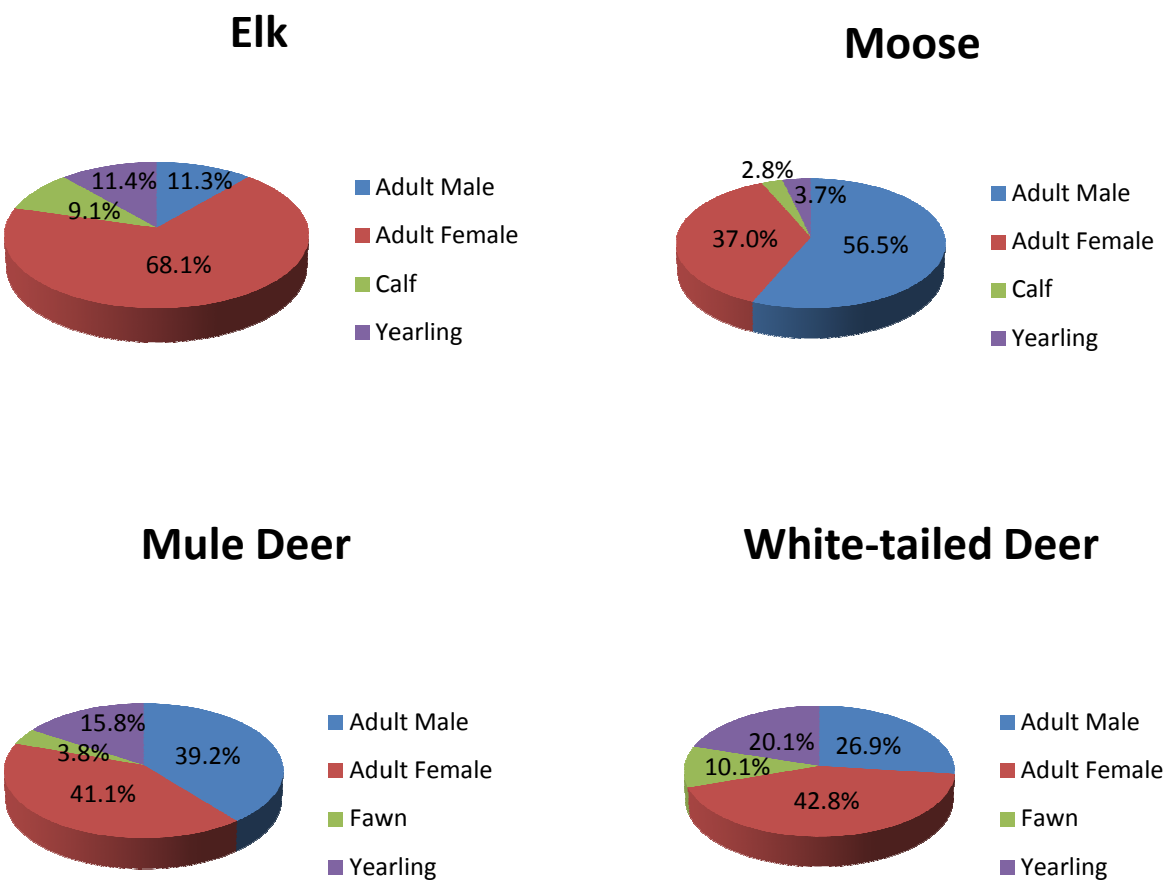
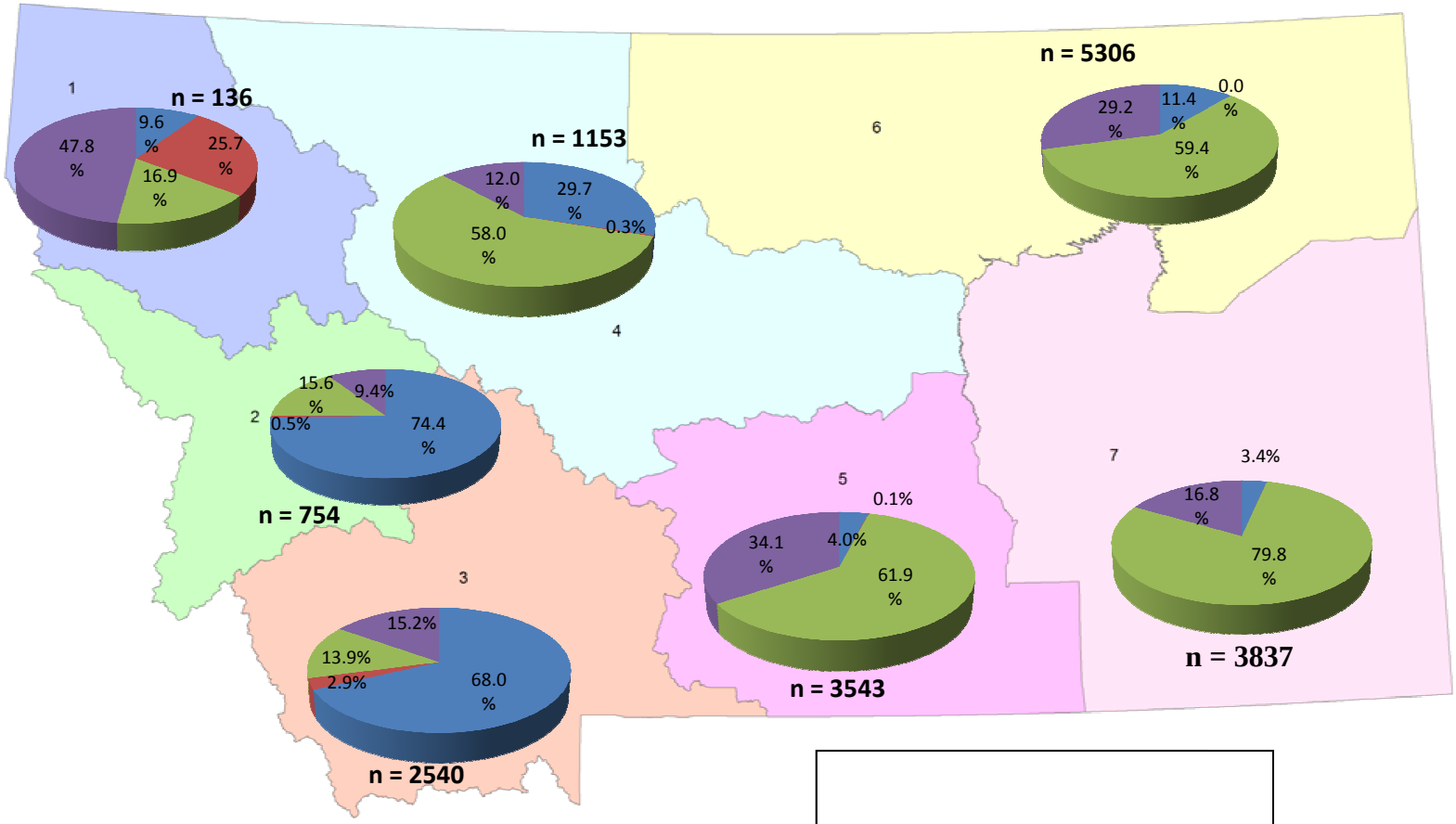



Figure 3. Age composition of elk, moose, mule deer and white-tailed deer tested for CWD in Montana, 1998-2011. Adults (> 1 year of age) were divided into adult male and adult female categories.


1
n = 136
9.6 %
25.7 %
47.8 %
16.9 %
n = 1153
12.0 %
29.7 %
0.3%
58.0 %
6
n = 5306
29.2 %
11.4 %
0.0 %
59.4 %
4
2
15.6 %
9.4%
0.5%
74.4 %
n = 754
5
0.1%
34.1 %
4.0%
61.9 %
n = 3543
7
3.4%
16.8 %
79.8 %
n = 3837
3
15.2%
13.9%
2.9%
68.0 %
n = 2540

2011.

Table 5. Special projects or collections

| Season | Location | Species | Status | Activity | Number |
|---|---|---|---|---|---|
| 1998 | Fort Keogh | Elk | Captive | Mortality | 1 |
| 2000 | Near Philipsburg, MT | Mule Deer | Wild | Removal of wild deer and elk near CWD positive alternative livestock facility | 9 |
| 2000 | Near Philipsburg, MT | Elk | Wild | Removal of wild deer and elk near CWD positive alternative livestock facility | 1 |
| 2000 | Elk Valley | Elk | Captive | Depopulate alternative livestock facility - contact herd with Philipsburg herd | 29 |
| 2000 | Sunlight | Mule Deer | Captive | Depopulate alternative livestock facility - contact herd with Philipsburg herd | 34 |
| 2001 | Ft. Peck - Corps of Engineers | Mule Deer | Captive | Removal of deer from elk observatory | 2 |
| 2001 | Ft. Peck - Corps of Engineers | White-tailed deer | Captive | Removal of deer from elk observatory | 8 |
| 2003 | National Bison Range | Elk | *Wild | CWD Surveillance | 52 |
| 2003 | Judith River | Elk | Captive | Escapees from alternative livestock facility | 12 |
| 2003 | Near Bozeman, MT | Fallow Deer | Captive | Depopulate alternative livestock facility - going out of business | 32 |
| 2005 | National Bison Range | Elk | *Wild | CWD Surveillance | 4 |
| 2005 | National Bison Range | Mule Deer | *Wild | CWD Surveillance | 1 |
| 2005 | National Bison Range | White-tailed deer | *Wild | CWD Surveillance | 4 |
| 2006 | National Bison Range | Elk | *Wild | CWD Surveillance | 1 |
| 2006 | National Bison Range | Mule Deer | *Wild | CWD Surveillance | 1 |
| 2006 | National Bison Range | White-tailed deer | *Wild | CWD Surveillance | 1 |
| 2007 | Beaverhead County | Elk | Captive | Escapees from alternative livestock facility | 2 |
| 2008 | National Bison Range | Mule Deer | *Wild | CWD Surveillance | 1 |
| 2009 | Wyoming | Elk | Wild | CWD Surveillance | 1 |
| 2009 | Wyoming | Mule Deer | Wild | CWD Surveillance | 1 |
| 2009 | Alberta, Canada | White-tailed deer | Wild | CWD Surveillance | 1 |
| 2010 | Utah | Elk | Wild | CWD Surveillance | 1 |
| Total | | | | | 199 |

## Discussion

The number, species composition, and age structure of animals tested for CWD varied by season and location within the state. Species composition of samples mirrored cervid densities and hunter harvest for given geographical areas. Within the eastern portion of Montana, mule deer were the predominant species tested; elk comprised the majority of samples in southwestern Montana, and white-tailed deer in the northwest. Survey design did not target region 1 in northwestern Montana as it was not considered to be a high risk area, limiting the number of samples collected. Hunting districts near the CWD positive Philipsburg alternative livestock facility in Region 2 were a focus of surveillance from 2000 thru 2003. However, wild cervid densities near the alternative livestock facility were considered to be low, and surveillance was unable to detect evidence of CWD in wild populations. As a result, CWD surveillance utilizing hunter-harvested and road-killed animals was discontinued in region 2 in 2004. Hunting districts near the Montana border within regions 3, 4, 5, 6 and 7 became the priority areas for surveillance. Sample sizes within these areas reflect the changing surveillance goals..

Surveillance efforts focused on collecting adult ($\geq$ 2 years of age) animals with emphasis on collecting males when possible. Adult males were considered important for sampling as they have been reported to have a higher prevalence of CWD than adult females (Wolf et al. 2004, Grear et al. 2006), and CWD prevalence in adults was greater than that of yearlings (Miller and Conner 2005) in wild populations. However, adult females comprised the majority of the elk, mule deer and white-tailed deer sampled in Montana, primarily due to availability and reluctance of hunters to voluntarily give up heads from adult males. Adult males did comprise at least 1/4$^{th}$ of the samples tested for both mule deer and white-tailed deer. Efforts to collect road-killed elk, deer and moose increased when it was determined that animals involved in vehicle collisions had a higher prevalence than other portions of CWD affected populations (Krumm et al. 2005).

The recommended surveillance strategies and those employed by MFWP utilized convenience samples (hunter-harvested animals and road kills) as they were a cost-effective way to maximize total numbers of samples collected. However, evaluating the overall effectiveness of surveillance strategies relying on convenience samples to detect disease in free-ranging populations is often difficult. Convenience samples are not randomly distributed and may have biases for age and sex classes. Hunters may also select against symptomatic animals, as suggested in MFWP's hunting regulations, although Conner et al. (2000) suggested that preclinical or deer in early stages of CWD may be more vulnerable to harvest. In endemic CWD areas, the majority of CWD infected deer and elk detected in surveillance have been subclinical (Miller and Williams 2002). Although detection of clinical CWD infected animals is rare and less than ½ of the symptomatic animals in endemic areas have CWD (Williams 2005), surveillance programs that target symptomatic animals may be more effective than utilizing hunter-harvested samples alone (Williams et al. 2002). Many states, including Montana, have struggled with obtaining adequate sample sizes when relying on hunter-harvest as a primary collection method.

Additionally, diseases like CWD are not often evenly distributed but produce clusters or pockets of infected animals within a population (Nusser et al. 2008). Although road-killed deer may have a higher incidence of CWD than the remaining population in CWD infected areas

(Krumm et al 2005), the distribution of road-kills may not overlap CWD disease clusters, particularly in rural areas with low road densities. Targeted surveillance of symptomatic animals can be an effective way to detect new foci of disease, but in the relatively remote areas of Montana potential CWD cases can go unobserved or unreported. Deer and elk demonstrating CWD symptoms are typically in the final stages of the disease (Williams 2005), indicating that it may have been present in the population for some time. Although the $PrP^{cwd}$ can be detected in elk and deer tissues of preclinical animals, convenience sampling alone may not adequately sample the population to detect CWD at low levels (Walsh and Miller 2010, Walsh 2012)

In order to improve surveillance efficiency, reduce the number of samples collected, and maintain high confidence of detecting the disease, Walsh and Miller (2010) and Walsh (2012) suggest employing a weighted surveillance strategy. The strategy utilizes a tiered approach but puts additional weight on collecting animals that have a higher likelihood of being CWD positive. For instance, CWD prevalence was nearly 10 times higher for symptomatic mule deer than what was observed in hunter harvested deer in Colorado (Walsh and Miller 2010). Krumm et al. (2005) suggested that road-killed mule deer in CWD endemic areas are nearly twice as likely to be CWD positive, compared to randomly sampled herd mates. Adult males tend to have higher incidence of CWD infection than adult females (Walsh and Miller 2010 and others). Walsh and Miller's (2010) weighted surveillance approach assigned a value or "score" to the type of samples collected (symptomatic animal, road kill, hunter harvest etc) based on the likelihood of it being CWD positive. The goal of the surveillance was to reach a certain score rather than some minimum number of samples. If more "high-scoring" animals are sampled, fewer overall samples are needed within a given area, and the overall cost of surveillance is lower.

Further adapting the weighted surveillance approach, Walsh (2012) recommends a surveillance strategy that incorporates both spatial and demographic risk factors with a weighted surveillance system in an effort to maximize cost efficiency and improve the probability of detecting CWD in systems where it has yet to be detected. MFWP is currently working with Robin Russell from the USGS on a model to help identify areas at "high risk" for CWD in Montana based on spatial and demographic factors. Information from previous surveillance efforts in Montana, the model being developed in conjunction with USGS, and recommendations based on research from states with CWD in wild populations can be used to develop a weighted surveillance system for Montana.

Currently, no effective or cost-efficient ante mortem test is available for CWD. Collection of lymph tissue through tonsil biopsies in mule deer (Wolfe et al. 2004) and rectal lymph tissue in elk (Spraker 2006) are potential means of collecting samples from live animals. However, these techniques require the capture and handling of deer and elk, which is typically cost prohibitive. These methods could be utilized in conjunction with other research activities that require the handling of animals. The detection of $PrP^{cwd}$ in feces of infected deer and elk, and the recent use of highly sensitive tests to detect $PrP^{cwd}$ of naturally infected elk (Pulford et al. 2012) suggest that non-invasive surveillance techniques may be available in the future. Until those methods are available, the testing of dead animals will be necessary for conducting CWD surveillance in free-ranging wildlife.

Although CWD has been found near the Montana border in North Dakota, South Dakota, Wyoming, Alberta and Saskatchewan, the prion associated with CWD has yet to be detected in free-ranging wildlife populations (or alternative livestock operations in Montana since initially being detected in the Philipsburg facility). The finding of no evidence of CWD is encouraging, but does not preclude the possibility that surveillance was unable to detect CWD at low prevalences in free-ranging wildlife populations. With the reduction of funding for CWD surveillance, a new strategy for the early detection of CWD will have to be developed should MFWP continue to look for the disease. Implementation of this new strategy will likely require additional support from regional wildlife and enforcement staff. Time constraints for existing regional staff and funding limitations should be considered when designing future surveillance strategies.

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