Feasibility of "test-and-cull" for managing chronic wasting disease in urban mule deer

Lisa L. Wolfe, Michael W. Miller, and Elizabeth S. Williams

Abstract　Strategies for managing chronic wasting disease (CWD) in urban mule deer (*Odocoileus hemionus*) populations are needed in Colorado. We captured, tonsil-biopsied, marked, and tested adult mule deer ($n=181$) to evaluate the feasibility of conducting an urban "test-and-cull" program in Estes Park, Colorado. During December 2002 and April–May 2003, we successfully tested 51 (57%) of the estimated 89 male and 130 (50%) of the estimated 261 female mule deer wintering in Estes Park for evidence of CWD. Nine (18%) males and 6 (5%) females tested positive for CWD infection via immunohistochemistry. All 15 test-positive deer were removed from the population; we culled 13 of these, and 2 died of other causes before being culled. We completed all sampling and inventory work in 34 field days and culled all test-positive deer in another 7 field days. Personnel time associated with sampling, culling, and inventory averaged 5.2 person-hours/deer. Average drug costs varied by combination (tiletamine–zolazepam–xylazine: $22; thiafentanil– xylazine: $66). Additional fixed costs averaged $215/deer for telemetry devices and $60/deer for vehicle and other testing-related expenses. Based on our initial assessment, sampling $\geq 50\%$ of the mule deer in Estes Park annually is feasible.

Key words　chronic wasting disease (CWD), epidemiology, mule deer, *Odocoileus hemionus*, prion, urban wildlife management

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE) of native North American deer (*Odocoileus* spp.) and elk (*Cervus elaphus nelsoni*) (Williams and Young 1980, 1982; Williams and Miller 2002). Areas of relatively high CWD prevalence in mule deer (*O. hemionus*) are associated with urban areas along Colorado's northern Front Range (Miller et al. 2000, Wolfe et al. 2002), and strategies for managing CWD in urban deer populations are needed if overall disease management objectives of lowered prevalence (Colorado Wildlife Commission 2002) are to be met. Although hunting has been identified as the preferred means of controlling CWD in Colorado (Colorado Wildlife Commission 2002), this strategy has limited application in controlling most urban

and suburban deer populations along the northern Front Range.

Epidemic models (Barlow 1996, Gross and Miller 2001) suggest that selective culling may be more effective than random culling (e.g., harvest) when a large proportion (>50%) of the population can be tested reliably and when infectious individuals can be removed relatively early in the disease course (Figure 1). Tonsil immunohistochemistry (IHC) is a sensitive and specific test for CWD in mule deer (Miller and Williams 2002, Spraker et al. 2002). Because disease-specific protease-resistant prion protein (PrP^{CWD}) accumulates in tonsils relatively early in the course of CWD infection in mule deer (Sigurdson et al. 1999, Miller and Williams 2002), tonsil IHC detects a high proportion of the infected

Address for Lisa L. Wolfe and Michael W. Miller: Colorado Division of Wildlife, Wildlife Research Center, 317 West Prospect Road, Fort Collins, CO 80526-2097, USA; e-mail for Wolfe: lisa.wolfe@state.co.us. Address for Elizabeth S. Williams: Wyoming State Veterinary Laboratory, Department of Veterinary Sciences, University of Wyoming, 1174 Snowy Range Road, Laramie, WY 82070, USA.
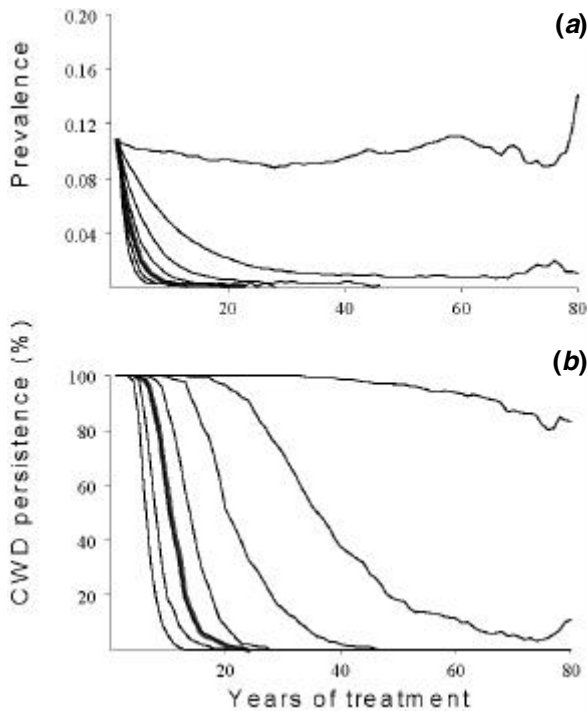
Figure 1. Simulated effects of test-and-cull management on (a) mean prevalence and (b) persistence (% of 200 simulations in which CWD was present at time *t*) of chronic wasting disease (CWD) in mule deer populations. Simulations assumed initial prevalence of 0.1, and testing applied to 10% (top or right-most line in each graph) to 70% (bottom or left-most line in each graph) of the population annually. The proportion of the population "tested" (Gross and Miller 2001) was assumed to be a product of sampling effort (proportion of population sampled) and test sensitivity (Miller and Williams 2002, Wolfe et al. 2002). Heavy line represents the proposed testing level for this study. Original base figure reprinted with permission from Gross and Miller (2001).

Study area

We evaluated the feasibility of a test-and-cull strategy for managing CWD using a naturally infected, free-ranging, urban mule deer population wintering in Estes Park, Colorado. We focused on mule deer wintering in urban areas, and consequently worked in areas where elevations ranged from 2,300–2,750 m. Our study area encompassed urban landscapes developed within native landscapes ranging from dense stands of mountain mahogany (*Cercocarpus montanus*) interspersed with grassland openings and small timbered patches of ponderosa pine (*Pinus ponderosa*) to mountain shrub habitat with a ponderosa pine and Douglas-fir (*Pseudotsuga meziesii*) overstory. Mule deer wintered throughout the study area, often close to human dwellings.

Methods

Based on model projections (Gross and Miller 2001; Figure 1), we wanted to evaluate the feasibility of applying a 50% "testing level" to the Estes Park deer herd. This "testing level" is a product of the proportion of the population sampled and the proportion of samples that have a sufficient number of follicles to detect CWD; for tonsil biopsy, on average about 90% of the samples are usable (Wolfe et al. 2002). It follows that a 50% testing level would be effected by sampling ≥55% of the adult (>1-year-old) deer population annually. Based on mark–resight inventory data from winter 2001–2002 (M. M. Conner and M. W. Miller, Colorado Division of Wildlife, unpublished data), we preliminarily estimated that 300–350 adult mule deer would be residing in Estes Park during winter 2002–2003 (November–May). We planned to use 100 drop-off radiocollars (Lotek Wireless, Newmarket, Ont.) to mark and test ≥175 adult mule deer wintering in Estes Park, dividing efforts between 2 rounds of capture and sampling.

We captured and sampled 113 adult mule deer during December 2002. Prior to release, each animal was marked permanently with ear tags and temporarily with a drop-off radiocollar set to release in March 2003. During January 2003 test-positive deer were located, recaptured, and euthanized; we also conducted mark–resight surveys to estimate population size for the Estes Park deer herd during January 2003 using established procedures (Bowden and Kufeld 1995, White 1996).

subpopulation (Miller and Williams 2002). Tonsil biopsy IHC is a reliable preclinical test for CWD in live deer that can be used under field conditions (Wolfe et al. 2002).

We describe results from a study evaluating the feasibility of conducting an urban "test-and-cull" program in Estes Park, Colorado. In this feasibility study, we attempted to capture, tonsil-biopsy, and mark adult mule deer in numbers approximating those needed to manage CWD via selective culling (Gross and Miller 2001). The specific objectives of this study were to assess the feasibility and costs of conducting test-and-cull management of CWD in an urban mule deer population and to continue evaluating and refining tonsil biopsy as a tool for managing CWD in free-ranging mule deer.

During March 2003 we recovered, cleaned, and refurbished dropped collars for use in the second field season. We captured and sampled another 88 adult mule deer during late April–May 2003 as described, and recaptured and euthanized test-positive deer by the end of May.

We captured mule deer and sampled them throughout Estes Park. We drove through neighborhoods to locate groups of deer (typically <10 animals) on properties where access permission had been secured. When we found deer on a parcel where we did not already have landowner permission, we either contacted the landowner before initiating capture or passed on the group. Because most parcels were relatively small residential lots (<1 ha), we were sometimes able to follow a group of deer until they moved onto an accessible property, or we returned to an area later to find deer on an accessible property. Where landowners could not be contacted, we left self-addressed, stamped postcards explaining our project and asking for permission to work on their property; we added returned cards that granted permission to our list of accessible properties.

We captured all deer via chemical immobilization from vehicles or on foot. We darted adult deer from groups until the group moved away or all untested adult animals had been caught and sampled. During the first round of testing, we refrained from capturing deer wearing collars placed previously for use in mark–resight inventories because most of these deer had been sampled <1.5 years ago; however, in the second round we did opportunistically capture and retest some of these deer. We used combinations of either tiletamine hydrochloride [HCl] and zolazepam (Telazol®, Fort Dodge Animal Health, Fort Dodge, Ia.; 250 mg/deer) and xylazine HCl (200 mg/deer) or thiafentanil oxalate (Wildlife Pharmaceuticals, Fort Collins, Colo.; 10 mg/deer) and xylazine HCl (100 mg/deer) delivered intramuscularly (IM) via projectile syringes fired from an adjustable, air-powered rifle (DanInject™; Wildlife Pharmaceuticals, Fort Collins, Colo.). Where appropriate, we used intravenous (IV) antagonists to reverse anesthetic effects after handling and sampling; for xylazine, we used tolazoline HCl (300–400 mg IV); for thiafentanil, we used naltrexone HCl (100 mg IV). We used Telazol and xylazine for the majority of captures, but switched to thiafentanil and xylazine for capturing females in late gestation.

Deer not completely anesthetized during capture received xylazine HCl (50 to 100 mg IV) as needed to facilitate muscle relaxation prior to sampling. Sampling techniques were as described by Wolfe et al. (2002). Once a deer was anesthetized, we placed a sliding gag (Design Metal Manufacturing, Fort Collins, Colo.) into its mouth and used a Mini Maglite® flashlight (Mag Instruments, Ontario, Calif.) to illuminate tonsillar crypts. We biopsied tonsillar tissue using a 30-cm rectal forceps with a 6-mm cup (Sontec Instruments, Denver, Colo.), and began taking sample bites at the rostral rim of the tonsillar sinus. We evaluated biopsy sites visually for bleeding or other complications after sampling was complete and applied gel foam to aid hemostasis. We preserved extracted tonsillar tissue in 10% neutral buffered formalin for histological evaluation.

We examined tonsillar biopsies via IHC (Miller and Williams 2002, Wolfe et al. 2002) using monoclonal antibody (MAb) F99/97.6.1 (VMRD, Pullman, Wash.). We evaluated biopsies microscopically for presence of lymphoid follicles and recorded the number of follicles. We further evaluated biopsies containing >1 lymphoid follicle for the presence of IHC staining in follicles and categorized as CWD-positive or -negative based on staining.

We tabulated test-positive and -negative results and used them to estimate local CWD prevalence as a baseline for future comparisons. We also recorded data on the performance of drop-off collars and tracked and calculated labor and operating costs (total, mean/animal tested) associated with our test-and-cull strategy. Operating costs included vehicle lease, mileage fees, drugs, darts, disposable medical equipment, radiocollars, drop-off devices, ear tags, and laboratory fees.

Results

Based on data from mark–resight inventories conducted during January 2003 (estimated population size 456, 95% CI = 416–500), about 350 adult (>1 year old) mule deer (89 male and 261 female) were estimated to have been residing in Estes Park during our study period (M. M. Conner, Utah State University, personal communication). During December 2002 and April–May 2003, we captured and sampled 201 adult mule deer wintering in Estes Park; the 56 males and 145 females that we sampled represented about 63% and 56% of the males and females that were estimated to be present in January 2003. By the end of this feasibility study, we

had secured permission from 705 landowners (97% of contacts) to capture deer on their respective properties.

Of the 201 samples collected, 20 (10%) were judged as unusable because no lymphoid follicles were observed microscopically. Consequently, 51 (57%) male and 130 (50%) female mule deer were successfully tested for evidence of CWD by IHC; in 140 (70%) of the usable samples, ≥ 9 follicles were present. Nine (18%) of 51 males and 6 (5%) of 130 females tested positive for CWD infection; overall, prevalence was 8% (95% CI = 4–12%). Prevalence among males was 3× greater than among females (Fisher's exact test $P = 0.013$). Of the 15 test-positive deer, we culled 13 from the population and 2 died of other causes before being culled.

We completed all sampling during 28 field days and removed all test-positive deer in 7 additional field days; we completed inventory in 6 field days. For all fieldwork associated with sampling, we averaged 5.2 person-hours per deer. Vehicle costs for all fieldwork averaged $28 per deer sampled. Average cost of drugs used in captures varied with drug combination used (Telazol-xylazine: $22; thiafentanil-xylazine: $66), including costs of missed darts, redarting, supplemental drugs, and antagonists. In addition to variable drug costs, fixed costs associated with testing were $32/deer, which included darts, ear tags, gel foam, penicillin, and laboratory fees. Because we needed to find and eliminate test-positive deer but did not want radiocollars on tested animals permanently, we spent $185 on VHF collars and $193 for timed drop-off devices to remove collars from test-negative deer. Although VHF collars can be reused, the drop-off devices were single-use, so we estimated that the cost of telemetry marking materials would approach $215/deer sampled over a 5-year management program.

Of the first 100 drop-off devices deployed in our study, 26 failed to release. Consequently, we incurred additional time and operating expenses recapturing collared deer to remove VHF collars for reuse, but we did not include these recaptures in calculating overall cost estimate because this appeared to be a one-time problem. Design modifications should remedy this problem, but continued failures at this rate could substantially increase the cost of using this strategy. Estimated costs also do not fully reflect the overall cost of this program because many of the landowner contacts were made during previous field studies in Estes Park; however, contacts made during this study are

included in the estimated labor for capture, inventory, and culling of positive deer.

Discussion

Based on our initial assessment, it appears that sampling at least half of the Estes Park deer herd annually is feasible, at a cost of roughly $300/animal plus personnel time (about 5 hours/animal, including inventory and culling of positives). However, we recognize that sampling the Estes Park mule deer population may be a somewhat unique situation. Although the size and behavior of this herd is representative of many other urban deer populations inhabiting Colorado's Front Range, urban white-tailed deer (*O. virginianus*) populations or mule deer populations in other areas may not lend themselves as readily to such approaches. The Estes Park deer were habituated to people and were readily captured with a dart gun (Figure 2). If deer were less approachable, the cost of personnel time and the feasibility of darting could be prohibitive. Costs also could be substantially greater in an area where landowner permission had not been secured previously or where landowners were uncooperative. Alternatively, in situations where drop-nets or Clover traps could be used to capture deer, overall costs of this management approach might be lower. Because conditions vary widely, "test-and-cull" CWD management may not be feasible in many areas; consequently, application of this approach to other

Figure 2. Estes Park mule deer were habituated to people and were readily captured with a dart gun. Here, a yearling male mule deer is tonsil-biopsied by Lisa Wolfe (right) and Michael Sirochman (left) while other deer stand in the background. This photo was taken in October 2003; all 7 deer in the background were captured and tested during the 2002 field season. Photo by M. W. Miller.

mule or white-tailed deer studies would need to be evaluated on a case-by-case basis.

Reducing CWD prevalence to our management goal of <2% via test-and-cull or any other conceivable management approach likely will take 5–10 years based on model projections (Gross and Miller 2001). Consequently, management programs undertaken to combat CWD should be regarded as long-term commitments of personnel and funding regardless of the approach taken. Given the success of our initial field study, we plan to continue to test and selectively cull animals that test positive from the Estes Park deer herd over the next 5 years to evaluate the effectiveness of this approach in reducing CWD prevalence. Because the Estes Park mule deer do not interact extensively with other CWD-infected population units in north-central Colorado (Conner and Miller 2004), prevalence responses should more closely approximate model projections than if Estes Park deer interacted extensively with other population units where prevalence was still high.

The 8% CWD prevalence estimated from deer sampled in our feasibility study was similar to prevalence reported previously in deer sampled in Estes Park (5%, 95% CI=2–10%; Wolfe et al. 2002). We observed a higher prevalence in male mule deer compared to females. A similar trend (prevalence in males about 2× prevalence in females) occurred in harvested deer in other populations near our study area in north-central Colorado (M. W. Miller, unpublished data). The mechanism underlying this disparity between the sexes is unclear. Males, through some aspect of behavior or distribution, somehow may be at higher risk of exposure to CWD. Because Estes Park and nearby Rocky Mountain National Park represent refuges from hunting, male mule deer appear to survive longer than in more heavily hunted populations. Miller et al. (2000) reported increasing prevalence of CWD in older age classes of male mule deer harvested in north-central Colorado. Greater longevity thus may account for the markedly higher prevalence rates that we observed in male mule deer in Estes Park. The mechanisms underlying these trends, as well as their management implications, may warrant further consideration and investigation.

Literature cited

BARLOW, N. D. 1996. The ecology of wildlife disease control: simple models revisited. Journal of Applied Ecology 33: 303–314.

BOWDEN, D. C., AND R. C. KUFELD. 1995. Generalized mark–resight population size estimation applied to Colorado moose. Journal of Wildlife Management 59: 840–851.

COLORADO WILDLIFE COMMISSION. 2002. Colorado Wildlife Commission Policy. Subject: Chronic Wasting Disease. http://wildlife.state.co.us/CWD/CWDPolicy_6-02.pdf (accessed 27 August 2002).

CONNER, M. M., AND M. W. MILLER. 2004. Spatial epidemiology in natural populations: a case study of movement and prion disease prevalence relationships among mule deer population units. Ecological Applications: in press.

GROSS, J. E., AND M. W. MILLER. 2001. Chronic wasting disease in mule deer: disease dynamics and control. Journal of Wildlife Management 65: 205–215.

MILLER, M. W., AND E. S. WILLIAMS. 2002. Detecting PrP^{CWD} in mule deer by immunohistochemistry of lymphoid tissues. Veterinary Record 151: 610–612.

MILLER, M. W., E. S. WILLIAMS, C. W. MCCARTY, T. R. SPRAKER, T. J. KREEGER, C. T. LARSEN, AND E. T. THORNE. 2000. Epizootiology of chronic wasting disease in free-ranging cervids in Colorado and Wyoming. Journal of Wildlife Diseases 36: 676–690.

SIGURDSON, C. J., E. S. WILLIAMS, M. W. MILLER, T. R. SPRAKER, K. I. O'ROURKE, AND E. A. HOOVER. 1999. Oral transmission and early lymphoid tropism of chronic wasting disease PrP^{res} in mule deer fawns. Journal of General Virology 80: 2757–2764.

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.

WHITE, G. C. 1996. NOREMARK: population estimation from mark–resighting surveys. Wildlife Society Bulletin 24: 50–52.

WILLIAMS, E. S., AND M. W. MILLER. 2002. Chronic wasting disease in deer and elk in North America. Pages 305–316 *in* R. G. Bengis, editor. Infectious diseases of wildlife: detection, diagnosis, and management. Revue scientifique et technique Office international des Epizooties 21.

WILLIAMS, E. S., AND S. YOUNG. 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. Journal of Wildlife Diseases 16: 89–98.

WILLIAMS, E. S., AND S. YOUNG. 1982. Spongiform encephalopathy of Rocky Mountain elk. Journal of Wildlife Diseases 18: 463–471.

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.

Lisa L. Wolfe (left) is a wildlife veterinarian with the Colorado Division of Wildlife's Wildlife Research Center. She obtained a B.S. in human nutrition, an M.S. in animal nutrition, and a D.V.M., all from Colorado State University. **Michael W. Miller** (right) is also a wildlife veterinarian with the Colorado Division of Wildlife's Wildlife Research Center. He obtained a B.S. in zoology (biochemistry minor), a D.V.M., and a Ph.D. in Wildlife Biology, all from Colorado State University. **Elizabeth S. Williams** is a wildlife pathologist and professor of veterinary sciences in the Department of Veterinary Sciences at the University of Wyoming. She obtained a B.S. in zoology from the University of Maryland, a D.V.M. from Purdue University, and a Ph.D. in veterinary pathology from Colorado State University.

Associate editor: *Krausman*