

## SPATIAL AND TEMPORAL DYNAMICS OF PRION DISEASE IN WILDLIFE: RESPONSES TO CHANGING LAND USE

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### PROJECT ACTIVITIES

Chronic wasting disease (CWD) of the deer family is a transmissible spongiform encephalopathy, a member of a group of infectious diseases, known as prion diseases, affecting animals and people. Similar diseases include scrapie in sheep and goats, bovine spongiform encephalopathy in cattle and Creutzfeldt-Jacob disease in humans. These diseases are associated with proteinase-resistant prion protein ( $\text{PrP}^{\text{res}}$ ) that accumulates in the brain of affected individuals, causing neural degeneration and eventually, death.

The only region in the world where prion diseases are known to occur in free-ranging animals is northeastern Colorado/southeastern Wyoming, where an epidemic of CWD has been ongoing for at least two decades. In some areas within this region, as many as 15% of sampled mule deer (*Odocoileus hemionus*) are infected with CWD. These naturally infected populations offer a unique opportunity to understand the transmission dynamics of a disease that has potentially enormous consequences for wildlife and that could threaten human health and economies over large areas. Although CWD is currently localized, similar diseases have global scope. It follows that limiting the spread of CWD represents a fundamentally important challenge for protecting the health of natural and human dominated ecosystems throughout the region, and in the fullness of time, throughout the world.

The spatial dynamics of this disease operate in an environmental context undergoing dramatic, human-induced change. The region where CWD is prevalent also contains one of the most rapidly growing human populations in the nation. Expansion of the human population is causing sustained changes in land-use and land cover; changes that perforate, compress, and fragment habitats of animals infected with CWD. These dynamics in landscape configuration are likely to shape spatial and temporal dynamics of the disease as infected populations respond to shrinking habitat and changes in sources of natural and human-caused mortality.

Our long-term goal is to understand and predict the spatial and temporal dynamics of CWD transmission. This goal includes three specific aims:

**Aim 1:** Describe mechanisms of CWD transmission between infected and susceptible individuals and determine if environmental sources of infectious prions ( $\text{PrP}^{\text{CWD}}$ ) can contribute to disease transmission.

**Aim 2:** Describe spatial and temporal variation in disease prevalence.

**Aim 3:** Select the best approximating models of disease dynamics and use these models to investigate anthropogenic effects of habitat compression and fragmentation resulting from sustained changes in human land-use.

To meet these objectives, we are conducting laboratory, field, and modeling studies. Laboratory studies will illuminate mechanisms of transmission and will provide a basis for formulating models. Field studies will assemble data for modeling spatial and temporal variation in the disease.

## **Activities Relevant to Aim 1: Describe Mechanisms of Disease Transmission**

### *Investigations of Shedding of PrP<sup>res</sup> and Vertical Transmission*

The purposes of this investigation are to determine whether mule deer shed infectious doses of PrP<sup>res</sup> in saliva, feces, urine, and/or reproductive tissues and fluids. Fawn-raising facilities and personnel have been established in Colorado and Wyoming. Arrangements have been made to acquire mule deer fawns to be hand-raised to serve as the PrP<sup>res</sup> inoculated animals from which excreta and tissues will be collected. Mule deer reproduction is highly seasonal and the fawning season begins in June. We expect to acquire the fawns needed for the study over the next several months. These fawns will be exposed to CWD agent later this summer.

We are in contact with two groups who have developed or are in the process of developing transgenic mice expressing the cervid PrP gene. Both groups are enthusiastic about collaborating on the proposed studies to assess PrP<sup>res</sup> shedding from experimentally infected deer. We expect these mice will be available within the year to begin breeding adequate numbers of mice for assaying excreta collected from the infected deer.

Because we believe that feces is likely to be a major route of excretion of the CWD agent, mule deer fawns are being hand-raised at a non-CWD endemic facility for exposure to feces from the experimentally inoculated deer. This will allow us to gather data on the potential for fecal shedding in advance of the results from transgenic mice.

### *Studies of Environmental Transmission*

The purposes of this investigation are to determine if indirect transmission of CWD occurs via exposure to environmental sources of PrP<sup>res</sup>. Arrangements have been made to acquire weaned mule deer fawns from non-CWD endemic areas later in the summer. Experimental facilities have been prepared including paddocks that previously held CWD-infected deer, paddocks where CWD-infected deer have been allowed to decompose for >6 months, and control paddocks which have never held CWD affected deer.

## **Activities Relevant to Aim 2: Describe Variation in Prevalence**

### *Sampling Infected Populations*

Our ability to describe spatial variation in disease depends on accumulating a large number of observations of disease state over space and time. We examined tissue samples from 1,729 mule deer and white-tailed deer collected between October 2000 and April 2001 as part of ongoing surveillance and management programs conducted annually by the Colorado Division of Wildlife. Of these, 356 were from populations where CWD has never been diagnosed and the remainder were from known CWD-endemic populations. We collected representative samples of medulla oblongata (sectioned at the obex), tonsil, and retropharyngeal lymph node tissues (as available) from each deer. Tissues were examined via immunohistochemistry (IHC) using monoclonal antibody (MAb) F99/97.6.1 (VMRD, Inc.) and staining techniques. Each tissue was evaluated independently for the presence of IHC staining and categorized as CWD-positive or -negative based on staining.

We are collaborating with the Wyoming Game and Fish Department to map data on location of CWD-positive and -negative deer samples tested since 1997.

In addition to these large-scale observations, we are observing fine-scale heterogeneity in disease prevalence with respect to contrasting patterns of land use within the epidemic area. We concentrated our efforts in the Estes Valley of Colorado where CWD prevalence is relatively high (8-12%). In particular, we selected individual mule deer in the city of Estes Park (EP) in Rocky Mountain National Park

(RMNP) for this study. The study was initiated Spring 2001 and is currently in progress. We determined that collecting 90 samples (tonsillar biopsies) from each land use type would allow us to detect differences of 1% between the urban and undeveloped land uses. From April 30 to May 31, we have sampled 41 individuals in EP and 21 in RMNP.

#### *Development of Sampling Techniques*

Current techniques for detecting CWD require killing animals, and this requirement limits our ability to conduct targeted surveillance without affecting abundance and distribution of animals. To overcome this limitation, we worked to develop novel non-lethal detection techniques. To that end, we examined tissue samples from 1,372 mule deer collected between October 2000 and April 2001 to compare sensitivity, specificity, and diagnostic agreement of tonsil and retropharyngeal lymph node IHC (which can be collected from live animals) to medulla oblongata IHC (which must be collected from culled animals). All samples were from harvested or culled free-ranging mule deer, with the exception of three captive individuals from the Colorado Division of Wildlife's Foothills Wildlife Research Facility; of the free-ranging samples collected, 290 were from populations where CWD has never been diagnosed and 1,079 were from known CWD-endemic populations). We collected representative samples of medulla oblongata (sectioned at the obex), tonsil, and retropharyngeal lymph node tissues from each deer. Tissues were examined via IHC using monoclonal antibody as described above. We estimated relative sensitivity, relative specificity, and agreement (kappa; Martin et al. 1987) of results from tonsil and retropharyngeal lymph node IHC using medulla oblongata IHC results as our diagnostic standard (Miller et al. 2000).

In addition, we began evaluating the practicality, utility, and reliability of using nonlethal diagnostic sampling to estimate CWD prevalence in a naturally-infected, free-ranging mule deer population. We are comparing CWD prevalence estimated from tonsillar biopsies to prevalence estimated from existing harvest-based survey data. Our study is designed to detect a meaningful difference in prevalence estimates derived from these two techniques. Based on preliminary data and our experience with CWD diagnostic techniques, it is unlikely that biopsy-based estimates will overestimate prevalence as compared to harvest-based estimates. Assuming that the latter prevalence estimate is about 0.1 (Miller et al. 2000, unpubl. data), and given the variation in prevalence observed annually, 50% differences in estimates between tests seem to us a reasonable benchmark for comparing techniques. Using sample size calculations based on effect size and 95% confidence intervals, biopsies from a random sample of 155 mule deer should be sufficient to detect a >0.05 underestimate of CWD prevalence. If we fail to detect such differences, then we will regard tonsillar biopsy-based prevalence estimates as equivalent to harvest-based estimates in future studies. Because we anticipate some proportion (~10%) of the biopsies may be regarded as inadequate for evaluation, we plan to capture and sample 170 adult mule deer in order to evaluate this technique for estimating CWD prevalence.

Finally, we improved the diagnostic capabilities for detection of PrP<sup>res</sup>. Two models of a rapid automated immunostainer have been brought on-line and we are getting reproducible results with nervous tissue, as well as with lymphoid tissues. This system has been employed in testing 2,385 deer samples from Colorado and Wyoming during the 2000-2001 CWD surveillance. In addition, this system works well for testing tonsil biopsies for evidence of PrP<sup>res</sup> accumulation. To date, 98 tonsil biopsies have been tested.

### **Activities Relevant to Aim 3: Select Models of Disease Dynamics**

#### *Developing Test Data Sets*

Selecting best approximating models of disease dynamics requires comparing model predictions of spatial and temporal variation in disease prevalence with empirical observations of prevalence. To make that comparison possible we began developing techniques for representing historic and current data on disease state based on hunter returns and culling. These techniques will produce a time series of maps (1996-

2005) portraying disease prevalence which can be used to: 1) test dynamic, ecological models of disease dynamics; 2) identify biases in hunter return data; and 3) develop statistical models explaining observed spatial variation. We are using a kernel density estimator (KDE) to approximate a continuous surface of disease prevalence based on point data on disease state (infected or not infected). A central challenge in using this method is to faithfully represent the relative influence of each point by selecting an appropriate smoothing constant, that is, a constant that is meaningful in the context of the biology and disease dynamics in mule deer. Our first exploration into this area focuses on estimates of individual and herd home range sizes as values for the smoothing constant.

#### *Data for Estimating Model Parameters*

Understanding patterns of movement is critical to parameterizing models representing spatial dynamics of CWD. In collaboration with the Colorado Division of Wildlife, we worked to describe patterns of mule deer movement and determine whether deer movements from areas of high CWD prevalence are related to levels of CWD prevalence in surrounding areas and/or mule deer populations. Secondly, we plan to use data from this study to assess relevant scales of spatial analysis and modeling. We believe that the area a single deer, or tightly associated group of deer, occupies during biological seasons may provide insight to the scale at which deer interact with CWD. Because dispersal and/or migration movements could be related to the spread of CWD, we focused our capture efforts on fawns and yearlings (6-18 months of age) during the first year of the study. Eighty mule deer were captured and radiocollared in two areas of high CWD prevalence during Winter 1999-2000. Radiocollared deer were located every one to two months from January 2000 to January 2001. During Winter 2000-2001, additional deer were captured to replace deer that died or dropped their radiocollars, bringing the total number of radiocollared animals back to 80. Deer radiocollared during the second year were captured primarily at the edges of the southern portion of the core CWD-endemic area. Deer were located monthly from January to May 2001.

We also assembled demographic data for a population in the approximate center of the epidemic area. The data span 1996-2000 and include estimates of adult and juvenile survival, recruitment, total numbers, and sex and age composition. For comparison, we obtained similar data for two populations known to be free of CWD. We estimated growth rates of the female portion of the population assuming a 50/50 sex ratio in fawns recruited to six months of age. Growth rates were estimated as the dominant eigenvalue ( $\lambda$ ) of the projection matrix. Confidence intervals on estimates of  $\lambda$  were estimated on bootstrapped samples drawn from four years of data on recruitment and adult and juvenile survival and recruitment to six months of age.

#### *Implementation of Spatial Models*

We began to implement numerical methods for modeling spatial variation in prevalence of CWD over time. We consider a rectangular region  $R$ . The region  $R$  will be some region including parts of northeastern Colorado and southeastern Wyoming. We then consider the following system of partial differential equations on  $R$

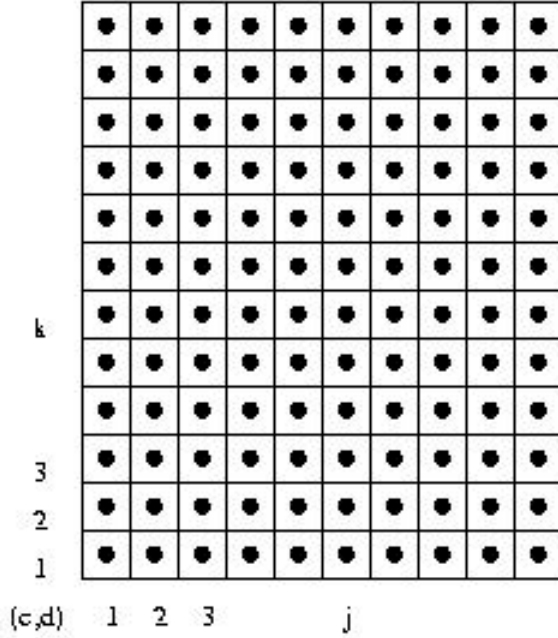
$$\frac{\partial s}{\partial t} = as + (1-m)aI - \beta \frac{sI}{N} - (b_s + h)s + \nabla(R^s \nabla s) \quad (1)$$

$$\frac{\partial I}{\partial t} = maI + \beta \frac{sI}{N} - (b_I + h)I + \nabla(R^I \nabla I), \quad (2)$$

where  $s=s(x,y,t)$  denotes the number of susceptible deer at point  $(x,y)$  and time  $t$ ,  $I=I(x,y,t)$  denotes the number of infected deer at point  $(x,y)$  and time  $t$ ,  $a$  represents the birth rate for deer,  $m$  is the percentage of fawns born to infected animals that are infected at birth,  $\beta$  is the transmission coefficient,  $b_s$  and  $b_I$  represent the death rates of susceptible and infected deer and  $h$  is the percentage of deer killed due to hunting. We refer to the two other functions  $R^s$  and  $R^I$  in the above equations as the porosity coefficients

for susceptible and infected deer. These functions will determine the movement and lack of movement of deer due to physical barriers that are present, altitude considerations, food available, etc.

We approximate the solution to the above system of partial differential equations by using finite difference techniques. We begin by placing a grid over the region  $R$  consisting of  $N$  equally spaced vertical lines over the region and  $M$  equally spaced horizontal lines as illustrated in Figure 1 below. We will use a cell centered approach and denote the rectangle to the right of the line  $y=c+j\Delta x$  and above the line  $x=d+k\Delta y$  as the  $j$ - $k$  th cell for  $j=0, \dots, M, k=0 \dots N$  (where  $(c,d)$  are the coordinates of the lower left hand side of the rectangle).



The approximations of  $s$  and  $I$ , written  $s_{jk}^n$  and  $I_{jk}^n$ , will represent the number of susceptible and infected deer in the  $j$ - $k$  th cell and will be considered as evaluated at the center of the cell (at  $(c + (j+1/2)\Delta x, d+(k+1/2)\Delta y)$ ) at time  $n$ . The finite difference approximation of equations (1)-(2) can then be written as

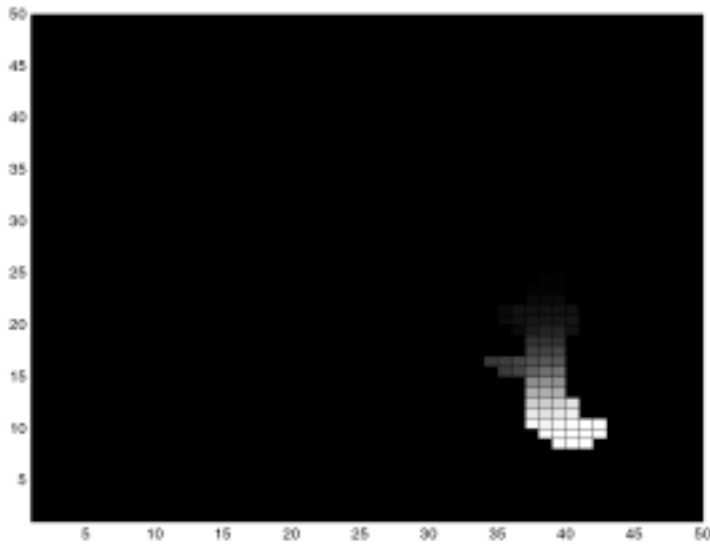
$$\begin{aligned}
 s_{jk}^{n+1} = & s_{jk}^n + a\Delta t s_{jk}^n + (1-m)a\Delta t I_{jk}^n - \beta\Delta t \frac{S_{jk}^n I_{jk}^n}{N_{jk}^n} - (b_s + h)\Delta t s_{jk}^n \\
 & + \frac{\Delta t}{\Delta x^2} \left[ R_{j+\frac{1}{2}k}^s (s_{j+1k}^n - s_{jk}^n) - R_{j-\frac{1}{2}k}^s (s_{jk}^n - s_{j-1k}^n) \right] \\
 & + \frac{\Delta t}{\Delta y^2} \left[ R_{jk+\frac{1}{2}}^s (s_{jk+1}^n - s_{jk}^n) - R_{jk-\frac{1}{2}}^s (s_{jk}^n - s_{jk-1}^n) \right]
 \end{aligned} \tag{3}$$

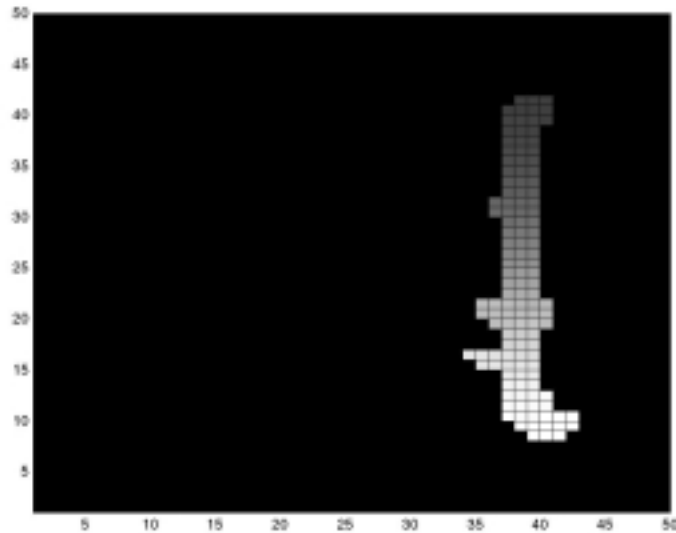
$$\begin{aligned}
I_{jk}^{n+1} = & I_{jk}^n + ma\Delta t I_{jk}^n + \beta\Delta t \frac{S_{jk}^n I_{jk}^n}{N_{jk}^n} - (b_s + h)\Delta t I_{jk}^n \\
& + \frac{\Delta t}{\Delta x^2} \left[ R_{j+\frac{1}{2}k}^l (I_{j+1k}^n - I_{jk}^n) - R_{j-\frac{1}{2}k}^l (I_{jk}^n - I_{j-1k}^n) \right] \\
& + \frac{\Delta t}{\Delta y^2} \left[ R_{jk+\frac{1}{2}}^l (I_{jk+1}^n - I_{jk}^n) - R_{jk-\frac{1}{2}}^l (I_{jk}^n - I_{jk-1}^n) \right]
\end{aligned} \tag{4}$$

We should notice that the equations can also be viewed as discrete equations representing birth, death, change from healthy to diseased and movement into and out of the  $j$ - $k$  cell.

The boundary conditions that we will use are no flow boundary conditions. We do this because we feel that we can choose a region that is sufficiently large enough that the boundaries are unimportant to the calculations. The way that we actually implement the no flow boundary conditions is by setting the appropriate  $R^s$ 's and  $R^l$ 's equal to zero near the boundary.

We have performed a test simulation with the understanding that the coefficients, porosity functions, etc., are not realistic. We actually choose  $a=m=b_s=b_l=h=0$ . The reason for this simulation is to illustrate that the mathematical model described above can model and control the movement of deer and disease. We used an initial distribution of susceptible deer that have a heavy concentration along a vertical strip and a light concentration elsewhere (a distribution that is at least similar to the front range of Colorado-Wyoming). We fix the porosity functions so that the animals are more apt to move along that same strip (where there is abundant food and little elevation change). We set a group of infected deer in one cell near the bottom of the given strip and watch the disease move northward (i.e., toward the top of the maps below---lighter areas indicate higher prevalence. Below we include two plots showing the distribution of diseased animals, one after 100 time steps and one after 1000 time steps. It should be noted that this is a totally artificial simulation. But for this simulation, it should be noted that there are no infected animals to the left or right of the vertical strip (the choice of  $R^l$  makes the infected animals not want to go in either of those directions).





## PROJECT FINDINGS

### Findings Relevant to Aim 2: Describe Variation in Prevalence

#### *Sampling Infected Populations*

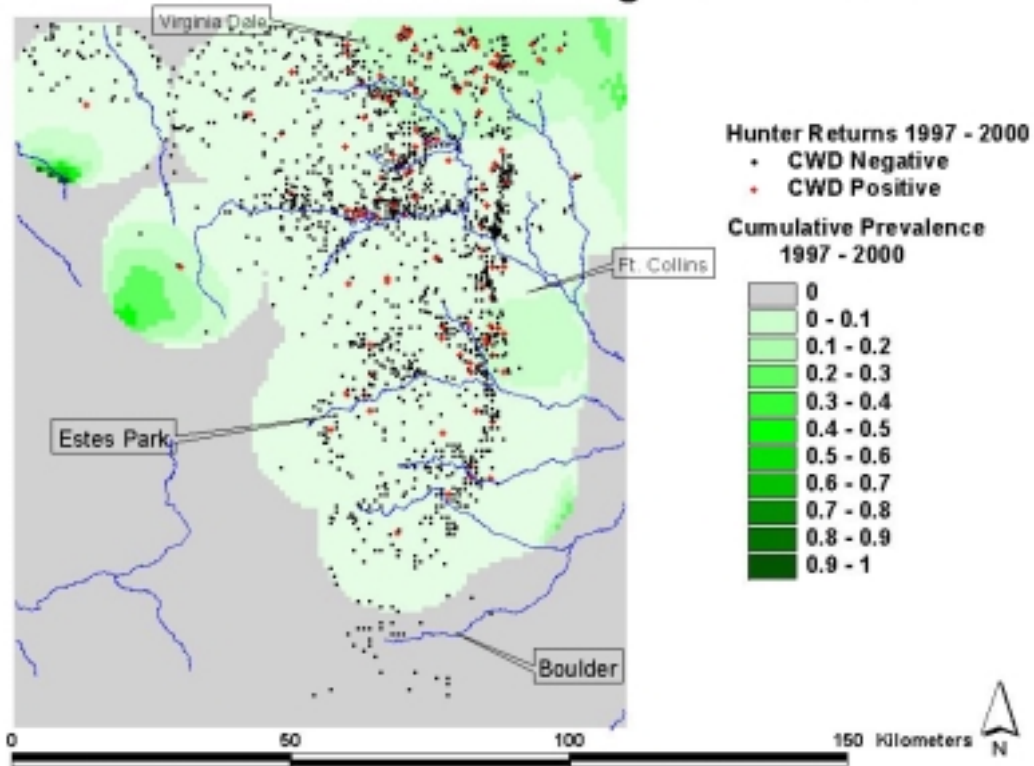
We selected a study area containing 98% of all known cases of CWD in mule deer. We used hunter return data to estimate average prevalence during 1997-2000 (Table 1) and to develop kernel density estimates (KDE) of spatial variation in prevalence across the study area (Figure 1). There is some evidence that CWD may be increasing based on the year 2000 data; average prevalence increased by more than 50%, although this increase may be due to chance alone. Moreover, refinement of detection techniques may explain at least part of this increase. Prevalence estimated with 15 km KDE appeared to be relatively homogeneous across the study area with most of the area showing prevalence of about 1%, with “hot spots” showing prevalence of 6-7% along the eastern and western borders of the study area. We emphasize that these patterns are highly dependent on choice of kernel size—for there are some game management units where CWD is known to exceed 15%. Work on evaluating the “best” kernel size is underway. Data on hunter returns from 1997 to 2000 showed no indication of increases in average prevalence (Figure 2).

**Table 1.** Prevalence of CWD estimated from hunter returns by year.

Year	Total Hunter Returns	Total CWD Infected Deer	Mean Prevalence	95% CI
1997	438	28	.064	.041-.087
1998	354	23	.065	.039-.091
1999	433	25	.058	.036-.080
2000	1002 <sup>1</sup>	56	.056	.042-.070

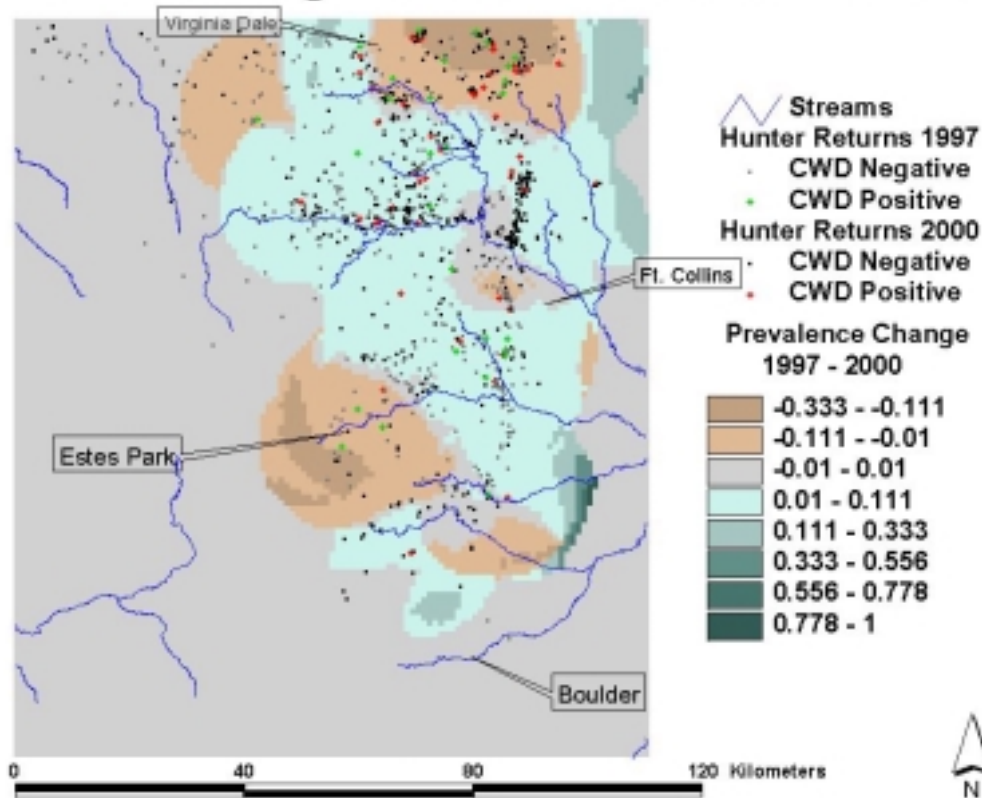
<sup>1</sup>Data from 2000 include culled animals as well as hunter returns.

## Prevalence in Mule Deer for All Hunter Returns Between 1997 and 2000 Using a 15KM KDE



**Figure 1.** Surface showing the CWD prevalence estimate based on all hunter returns collected between 1997 and 2000 using a 15 kilometer smoothing constant (KDE).

## Estimated Change in Prevalence 1997 - 2000



**Figure 2.** Surface depicting the change in CWD prevalence estimate between 1997 and 2000 within the spatial study area using a 15 kilometer smoothing constant (KDE).

### *Development of Sampling Techniques*

We found that samples of tonsil and lymph node tissue provide results that are very comparable to those traditionally obtained from the brain. Both tonsil and retropharyngeal lymph node IHC appeared to be highly reliable means of diagnosing CWD in mule deer. Using 74 medulla oblongata-positive deer as known infected individuals and the 290 deer from outside endemic areas as known uninfected animals, relative sensitivity of tonsil IHC was 97.3% (72/74) and relative specificity was 100% (290/290); similarly, relative sensitivity of retropharyngeal lymph node IHC was 98.6% (73/74) and relative specificity was 100% (290/290). Using data from all 1,372 sample sets, estimated kappas reflected a high degree of agreement between tests ( $\text{kappa}_{\text{medulla oblongata-tonsil}} = 0.94$ ;  $\text{kappa}_{\text{medulla oblongata-retropharyngeal lymph node}} = 0.94$ ;  $\text{kappa}_{\text{tonsil-retropharyngeal lymph node}} = 0.99$ ). Both tonsil and retropharyngeal lymph node IHC detected additional positive animals (7 and 8 cases, respectively). In light of geographic origins and high-test specificities, we regarded as CWD-infected animals not detected by medulla oblongata IHC. These findings are consistent with observations of positive IHC staining in lymphoid tissues (particularly retropharyngeal lymph nodes, tonsils, and mesenteric lymph nodes) in the absence of either lesions or staining in brain tissue in experimentally-infected mule deer and white-tailed deer. Based on our understanding of CWD pathogenesis, it appears likely that none of the available diagnostic tests for CWD can detect all infected animals in a population; CWD in its early stages remains undetectable. However,

our data suggest about 10% more truly infected deer may be detected in surveys using tonsil and retropharyngeal lymph node IHC compared with sampling brain tissue. One medulla oblongata IHC-positive deer stained negative on both tonsil and retropharyngeal lymph node IHC, suggesting some natural variation in CWD pathogenesis may occur among infected mule deer.

Field evaluation of tonsil sampling on live deer offered encouraging results. We needed to develop non-lethal techniques that provided at least one lymphoid follicle from each sample of tonsil tissue. The quality of samples was dramatically affected by sampling technique ( $P = 0.03$ ). Biopsy cup size (4 mm vs. 6 mm) appeared to have the greatest influence on number of follicles recovered -- using a biopsy forceps with a 6 mm cup consistently yielded more samples with follicles (27/39) than a 4 mm cup (6/25) across various visualization and site combinations. We attributed this difference to the ability to collect larger tissue pieces with the 6 mm cup. Manually holding the mouth open proved dangerous to the operator and instruments and was discontinued. Use of the swine vaginal scope was easy, provided good visualization of the sinus, and protected the biopsy instruments. However, usable sample yield (<40%) was much lower than when using the mouth gag (>71%). In retrospect, it appeared that the scope may have somehow distorted tonsillar tissue when pressed against the soft palate, thereby diminishing sample quality. The most consistent biopsy approach was taking the first bite at the rostral rim of the sinus, then rotating the biopsy forceps to take subsequent bites that included the sinus (20/21 samples with follicles, vs. 12/17 when sampling directly from the sinus; other variables held constant). Inserting the forceps directly into the sinus or attempting to take deep bites seemed to bypass follicular tissue altogether in many cases. Overall, by using a simple mouth gag and a 6 mm biopsy forceps, and by taking the biopsy starting at the rostral rim of the tonsillar sinus, 20/21 (95%) samples yielded at least one lymphoid follicle; moreover, most (18/20) of these samples yielded <sup>3</sup>3 follicles/sample, as recommended for antemortem diagnosis of scrapie in sheep.

Only a subset of samples collected to date have been examined by IHC, but our preliminary results appear promising: 1 of 27 (3.7%) biopsies from the EP area that have been examined thus far tested positive by IHC; the preliminary estimate of CWD prevalence based on tonsillar biopsies appears comparable to our most recent harvest-based prevalence estimate (5.5%; 7 of 126 positive) from that geographic area. Additional sampling and evaluation is ongoing. Successful development of this technique will radically change our ability to sample large numbers of animals from free ranging populations without altering their inherent dynamics.

### **Findings Relevant to Aim 3: Select Models of Disease Dynamics**

#### *Developing Test Data Sets*

We will use kernel density estimates of prevalence to test predictions of alternative models. Progress in developing these estimates was described above (see *Sampling Infected Populations*).

#### *Data for estimating model parameters*

Preliminary demographic analyses of data on a mule deer population where prevalence of CWD is relatively high (4-5%) cannot rule out declines in population numbers during 1997-2000 (Table 2). Although the population with CWD showed the lowest growth rate of the three examined, there was strong overlap among confidence intervals on  $\lambda$ , suggesting that while CWD is almost certainly having an impact on population growth, this impact cannot be meaningfully distinguished from other effects operating in uninfected populations (Table 2).

**Table 2.** Estimates of rates of growth of populations where CWD is known to be present and known to be absent (or undetectable).

Population	Prevalence of CWD	$\lambda$	95% CI on $\lambda$
DAU 4	4-5%	1.04	.96 - 1.11
DAU 19	0	1.07	.99 - 1.31
DAU 9	0	1.16	1.07 - 1.20

## **OPPORTUNITIES FOR TRAINING AND DEVELOPMENT**

At Colorado State University, we have staffed our project with two PhD students and a postdoctoral fellow. The post-doc is playing an important leadership role in the project and is gaining experience in management, as well as the scientific aspects of the project. The PhD students are an integral part of our team and are gaining unusual exposure to interdisciplinary work.

At the University of Wyoming, two undergraduate animal science/pre-veterinary science students are employed on the project to manage the experimental animals and to learn techniques for animal husbandry and infectious disease work. Another undergraduate student is hired in the histopathology laboratory and is assisting in processing tissues and immunohistochemistry. A Master's graduate student is participating in tissue sectioning in the histopathology laboratory and is learning immunohistochemistry. She recently completed the requirements and testing to become a certified histotechnologist in part based on work conducted in association with this study.

## **OUTREACH ACTIVITIES**

Hobbs and Williams gave lengthy interviews describing our project to the Science Reporter for the Wall Street Journal. Theobald gave an oral presentation and written project summary to the Institute on the Environment, a consortium of journalists including representatives from Audubon, Science and Technology News, and the Canadian Broadcasting Corporation.

We provided information for an article in the Estes Park Trail Gazette "Front Range Wasting Disease Targeted," June 6, 2001, to inform local citizens about work we were doing in their community. We also made many face-to-face contacts with landowners to gain permission to capture animals on private land. In all of these contacts, we distributed flyers describing the project.