

## Goals

Our long-term goal is to understand and predict the spatial and temporal dynamics of chronic wasting disease. 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 achieve these aims, we are conducting laboratory, field, and modeling studies. Laboratory studies will reveal 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 Transmission

#### *Investigations of Shedding of $\text{PrP}^{\text{res}}$ and Mechanisms of Lateral Transmission*

During summer 2001, 14 mule deer fawns were obtained as neonates from outside the CWD endemic area and hand raised in isolation facilities for use as “donor” ( $n = 7$ ) and “recipient” ( $n = 7$ ) animals for planned transmission studies. Another 10 mule deer and 2 white-tailed deer fawns from presumably unexposed herds were raised similarly during summer 2002 for use as recipients ( $n = 5+2$ ) and controls ( $n = 5$ ). Based on the original study plan, a third group of fawns may be raised in 2003 for use as additional recipients if needed.

In September 2001, seven of the 2001 cohort fawns were inoculated orally with 1 gram of CWD mule deer brain pool of known infectivity and housed at the Wyoming Game and Fish Department’s Sybille Wildlife Research Unit (Sybille). During April 2002, these donor fawns were immobilized and blood, saliva, feces, and urine were collected. Specimens were banked for use in assays for PrP and infectivity. These donor deer were sampled again in October 2002 and in February 2003 for collection of the same specimens. Additional feces were collected intermittently during April-February 2003 from donor deer. At least one of the inoculated donor deer was showing moderate clinical signs of CWD when sampled in February; we expect that the remaining donor deer will begin showing clinical signs of CWD over the next 6 months.

In order to determine if CWD is transmitted by feces via the presumed natural route of oral exposure, recipient mule deer fawns are being exposed to feces collected from the orally inoculated donor deer housed at Sybille (beginning with feces collected in February 2002). Recipient deer are housed in isolation outdoor facilities at the University of Wyoming (UW) facility in Laramie; controls are housed in outdoor isolation at the UW’s Red Buttes facility south of Laramie. The 2001 recipient deer were exposed over 8 separate periods between June 2002 and January 2003 to feces (about 35 g/period) collected from donor deer 7-12 months after inoculation (PI). The 2002 recipient deer were exposed over 7 separate periods between November 2002 and April 2003 to feces (about 47 g/period) collected from donor deer 13 months PI.

Transgenic mice expressing mule deer PrP are still under development at several institutions. One of our collaborators now has test mice inoculated with CWD material and is in the process of validating this transgenic system. When adequate numbers of appropriately validated mice are available to use for bioassay, we hope to inoculate them with materials collected from inoculated mule deer. Results

will be compared to data collected from direct deer challenges to assess the utility of such animal models for further study of CWD transmission.

Preliminary testing of specimens from experimentally inoculated deer has begun, but assays used to detect PrP<sup>CWD</sup> in tissue do not appear sensitive enough for use in excreta at this time. Technique modifications that may enhance assay performance are under evaluation (some of the following studies were conducted with partial funding from a variety of sources).

A potentially highly sensitive technique using surface enhanced raman immunoassay (SERIA) was evaluated for use in detecting CWD PrP<sup>CWD</sup> in tissues, excreta, and environmental samples. We found that assay components destabilized the colloids essential to the SERIA methodology and we will not pursue use of SERIA in the future. In addition, we worked with three commercial companies over the past 24 months to test the sensitivity, specificity, and efficiency of several ELISA tests for detection of PrP<sup>CWD</sup>. One of these tests (Platelia, BioRad, Hercules, California) was licensed by the Center for Veterinary Biologics (USDA) for use in CWD diagnostics in part using data generated in our laboratories. This competitive ELISA shows potential for detection of PrP<sup>CWD</sup> with high sensitivity (up to 0.2 mg starting tissue) and we have begun testing excreta for evidence of PrP<sup>CWD</sup>. A second test (dot-blot immunoassay, Veterinary Medical Research and Development [VMRD], Pullman, Washington) also was licensed by USDA following testing at our laboratories and elsewhere, but this test does not appear to have the necessary sensitivity to be useful in detecting PrP<sup>CWD</sup> in excreta and environmental samples and we will not continue work with this assay for this purpose. We are currently conducting assays with another ELISA (Enfur, Abbott Laboratories, Abbott Park, Illinois), but have not yet evaluated its sensitivity or its potential usefulness on excreta.

Techniques to improve sensitivity of our western blot detection of PrP were evaluated using several polyacrylamide gel electrophoresis (PAGE) formulations and detection systems. For PAGE, a Tris-glycine SDS discontinuous pH denaturing gel system after the method of Laemmli using either 10% or 12% acrylamide/bis-acrylamide separating gels with either 3% or 4% stacking gels was employed, in addition to the Bis-Tris [Bis(2-hydroxyethyl) imino-tris(hydroxymethyl) methane-HCl] neutral pH, discontinuous SDS-PAGE formulations of the NuPAGE Novex systems in preformed 10% or 12% polyacrylamide gels and either MOPS-SDS or MES-SDS running buffer. Gels were electrophoretically blotted onto PVDF membranes either in Towbin's buffer (25 mM Tris, 192 mM glycine, 20% methanol), or NuPAGE Transfer Buffer (25 mM Bicine, 25 mM Bis-Tris (free base), 1 mM EDTA, pH 7.2) with 10% methanol. Detection of bands reacting with murine anti-PrP monoclonal antibody F99/97.6.1 (VMRD) was tested with several systems also, including alkaline phosphatase (AP)-conjugated anti-mouse IgG secondary antibody followed by development with chromogenic AP substrate (NBT/BCIP); biotinylated secondary antibody followed by reaction with a streptavidin-AP conjugated biotin complex and chromogenic development with NBT/BCIP; two chemiluminescent detection systems: ECL which uses a horseradish peroxidase (HRP) conjugated secondary antibody against mouse IgG and a light-emitting substrate for HRP; and Tropix Western-Light Plus system which uses biotinylated secondary antibodies and AP-conjugated streptavidin and a luminescent substrate for AP.

In general all systems were found to work satisfactorily in the detection of PrP, although both the biotin-avidin system and the chemiluminescent systems had problems of non-specific background when exposure time was lengthened because of low intensity prion bands. Alkaline phosphatase chromogenic system was the least sensitive of those tested, although the cleanest. Quantitation studies have not yet been attempted. Additional techniques for improving sensitivity are being investigated for use in excreta and environmental samples.

### *Investigations of Environmental Transmission*

We continued an experiment comparing the relative contributions of live animals, contaminated environments, and infected carcasses to transmission of chronic wasting disease. Thirty-four free-ranging mule deer from two sources distant to known endemic foci of chronic wasting disease (Rocky Mountain Arsenal National Wildlife Refuge, US Air Force Academy, Colorado Springs, Colorado) were captured for use as experimental subjects during March-May 2002. We transported these deer to the Colorado Division of Wildlife's Foothills Wildlife Research Facility (FWRF), where they were placed in paddocks (n = 3 replicates/exposure route; n = 3 deer/paddock). Exposure treatments were: confinement in paddocks housing naturally-infected deer (1 infected deer/paddock), confinement in paddocks where infected deer previously resided, and confinement in paddocks where carcasses from CWD-infected deer have decomposed *in situ* (1 carcass/paddock); unexposed control paddocks are also being maintained. Entire paddock groups will be sacrificed and examined at the first sign of CWD in any subject deer within a paddock. We will compare infection rates, as well as time of onset, to gauge the relative contributions of these exposure sources to perpetuation of CWD epidemics.

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

#### *Sampling Infected Populations*

Surveillance for CWD continued in Wyoming during 2002. In collaboration with the Wyoming Game and Fish Department, over 2,550 specimens from mule deer, white-tailed deer, and elk were tested. Most samples came from the CWD endemic area. Prevalence and distribution remained approximately the same as in past years but a few new hunt areas, most not unexpected, are now included in the core endemic area. For the first time CWD was detected west of the continental divide in Wyoming. Maps of locations of samples collected during CWD surveillance were done.

The Colorado Division of Wildlife sampled over 26,000 deer and elk harvested or culled in northeastern Colorado and other select locations. Prevalence data from these samples will be available to augment the existing database that provides the foundation for our analysis and modeling of temporal and spatial aspects of CWD epidemiology. This year's data will be useful in further exploration of local patterns of disease prevalence related to deer densities and land use patterns.

#### *Development of Techniques for Sampling Live Animals*

In order to study CWD across landscapes where hunting and culling are not feasible sources of diagnostic samples, we developed techniques for sampling live animals. During the first two years of this project, we conducted a field study to evaluate tonsil biopsy immunohistochemistry (IHC) as a tool for diagnosing CWD in live, free-ranging mule deer (*Odocoileus hemionus*) and estimating prevalence. A small number of white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) have also been tested. Upon successful completion of our initial field study to refine and evaluate tonsil biopsy as a field tool for CWD work, we applied these techniques to gather data for new studies related to effects of land use patterns on CWD prevalence and its management.

#### *Local Scale Study: Effects of Land Use on Prevalence*

Over the last two decades, the Front Range of the Rocky Mountains has experienced rapid growth of its human population. Native plant communities and agricultural lands have been converted to residential and commercial development. Regional projections of human population growth suggest that these changes are likely to continue (Theobald et al. 2001). This same region is experiencing a protracted epidemic of chronic wasting disease (CWD) in free-ranging ungulates, an epidemic that has been in progress since the 1970's (Williams and Young 1992, Miller et al. 2000). Changes in land use shape the spatial distribution of prevalence of CWD as ungulate populations respond to habitat conversion by altering their movements. Changes in spatial distribution, in turn, may affect temporal

dynamics of the disease by altering rates of transmission.

We are working to understand the effect of land use change on CWD prevalence in free-ranging mule deer. As part of this effort, we conducted a study to determine whether CWD prevalence in urban areas is higher than in non-urban areas. Our study used prevalence data collected from adult mule deer across three sites in northern Colorado; Glacier View Meadows (GVM), Horsetooth (HT), and Estes Park (EP). At each of these sites we identified areas of urban development nested within a larger, non-urban region (Figure 1). We defined urban land use as areas containing  $> 1$  housing unit per 20 acres, while those with  $\leq 1$  housing unit per 20 acres were considered non-urban, typically containing privately owned ranches, as well as state and federal lands. Delineation of urban and non-urban regions was based on the most current map of housing density in northern Colorado (Theobald 2001).

Although samples of brains taken from hunter-returned deer heads have been used to estimate prevalence rates at these three sites since 1996, the data were insufficient to understand land use effects because most urban areas are not accessible to hunters. To obtain samples from urban areas we used tonsil biopsies (Wolfe et al. 2002) collected from randomly selected adult mule deer. In a previous study, it was shown that estimated prevalence rates were not significantly different between diagnoses made using either brain or tonsil tissue (Wolfe et al. 2002). Total sample size was 922 (Table 1), with slightly more samples coming from non-urban areas. Tonsil sampling was initiated April 30, 2001 and completed mid-May 2002. We developed a set of candidate models reflecting the probability of infection for each individual as a function of its sex and land use type, and site where it was sampled. Logistic regression was used to model the probability of infection as a function of these covariates, with land-use effects being of special interest. We then used  $AIC_c$  (Hurvich and Tsai 1989) to determine which models in the candidate set were supported by the data. Results from this research are being used to inform the landscape models that we are currently developing.

#### *Landscape Scale Study: Spatial Heterogeneity of CWD Prevalence*

To develop a dynamic, spatially explicit model of CWD requires an understanding of how features of the landscape contribute to the observed spatial heterogeneity in disease prevalence. Certain landscape attributes may serve as “basins of attraction” for CWD, concentrating and exacerbating its effects over relatively small spatial scales. Conversely, other landscape characteristics may act as barriers to transmission, effectively shielding a portion of the population from the disease, allowing some deer to remain relatively free from infection in both time and space. The overall goal of this component of our research is to understand and predict the spatial patterns and temporal dynamics of CWD transmission across the landscape. We foresee immediate application of this work in helping wildlife managers prioritize areas for control and surveillance.

During the past year, we initiated work using Bayesian hierarchical models that incorporate the spatial structure of the disease as a Markov Random Field (MRF) (Besag 1974, Künsch 1986, Besag et al. 1991, Cressie 1993, Bernardinelli et al. 1997). The MRF is represented by a grid structure laid over a map indicating the locations of infected and uninfected harvested deer. The MRF allows for the inclusion of spatial dependency in the model by assuming that prevalence of the disease in each grid-cell is determined, at least in part, by the spatial structure of CWD in surrounding grid cells, known as a neighborhood. Additionally, the Bayesian modeling framework provides several advantages over frequentist approaches. Using this approach, we are able to explicitly incorporate current knowledge about the biology and epidemiology of the system through the specification of prior distributions reflecting our understanding of the range of variability and functional form of model parameters, something that is impossible within a frequentist framework. Further, posterior model predictions are used as prior information to update the model as new data become available, resulting in a continuous dialogue between data and the model.

We chose to structure the spatial component of the model as a MRF based on our current understanding of mule deer biology, CWD aetiology, and the accuracy of the data collected. As noted

above, the spatial data on harvested deer are based on mapped point locations where deer were estimated to have been killed. These point locations are mapped by the hunters themselves. We know that these data are prone to location errors both due to the fact that deer move, and are thus unrealistically represented by a single point in space, as well as mapping errors introduced by the hunters. Because the MRF formulation is grid-based, we are able to aggregate individual deer samples into collections of deer at biologically relevant scales, thereby reducing location uncertainty while retaining biological realism. We have observed positive spatial autocorrelation in prevalence rates across the study area, which is precisely what one should expect from a disease that is transmissible either via environmental or animal-to-animal pathways. The MRF is designed to capture the component of variability that is due to the spatially correlated nature of the sample. Specifically, the spatial component of the Bayesian model is cast in terms of a Conditional Autoregressive (CAR) framework (Besag et al. 1991, Besag and Kooperberg 1995, Mollie 1996), where the infection rate of the disease within a grid-cell is explicitly modeled as a function of the infection rates within the local neighborhood. A variety of neighborhood structures will be considered with posterior distributions of all modeled parameters estimated using Markov chain Monte Carlo (Geman and Geman 1984).

Given our poor understanding of the dispersal range of this disease, i.e., the geographical range of influence exerted by disease cases on healthy individuals, our approach must necessarily model the spatial structure of CWD at several biologically relevant scales. One scale is the estimated home range size of wintering individuals. Based on radio-collared deer that have been tracked for the past 5 years the estimated home range size of 9 km<sup>2</sup> (Conner 2002) will be the first scale of disease influence to be modeled. A multi-scaled approach will allow us to determine which scale possesses the greatest likelihood for explaining the spatial dynamics of CWD. That is, by comparing the model's predictions to the observed data we will be able to determine quantitatively which scale produces the best fitting model, and infer from this the scale of management activities that should be most effective in reducing the spread of CWD. This determination will potentially provide insights not only into the dispersal capabilities of infected individuals but also into the most likely mechanisms of long-range transmission of the disease (e.g., individual versus herd migration patterns).

We concluded that a Bayesian hierarchical model incorporating an autoregressive component is a natural approach to this problem given the ability of the model to simultaneously capture the spatial structure of this disease's aetiology at biologically relevant spatial scales and model the contributions made by landscape features in shaping the observed spatial distribution of CWD. The model remains sufficiently flexible to be easily applied to our rapidly growing data set of spatial locations of infected and uninfected individuals.

#### *Evaluation of an Urban CWD Management Strategy*

We also assisted the Colorado Division of Wildlife in initiating a pilot study to evaluate a "test and cull" strategy for managing CWD in urban habitats. Models exploring probable consequences of various management strategies identified selective removal of infected individuals as a potentially effective method for reducing CWD prevalence in mule deer populations, provided that infected deer were detected early and a large (>50%) proportion of the population could be sampled annually (Gross and Miller, 2001, *J. Wildl. Manage.* 65:205-215). During November-December 2002, 113 free-ranging mule deer were captured, tested, and marked with timed-release radiocollars in urban areas throughout Estes Park to assess the feasibility of such a management approach. This sampling effort represented testing of about 25% of the adult mule deer residing Estes Park (M. Conner, personal communication). In January 2003, biopsy-positive deer were culled. Dropped radiocollars were recovered in March-April 2003 for reuse in a second round of sampling planned for April-May 2003. In addition to the primary goal of assessing feasibility, data gathered in the course of this study will also be useful in improving our understanding and modeling of the influences of urban landscapes on CWD epidemiology.

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

We used data from two epidemics of chronic wasting disease at the Foothills Wildlife Research Facility near Fort Collins, Colorado (Figure 2) to fit compartment models representing dynamics of transmission. Our objectives were to estimate transmission rates, to select best approximating models of disease dynamics, and to use best approximating models to gain insight into the behavior of the disease in natural populations.

#### *Candidate Models*

We evaluated four candidate models representing alternative hypotheses on disease states and routes of transmission. All models predicted cumulative mortality from CWD using linked differential equations portraying the number of infected ( $I$ ), susceptible ( $S$ ), and latent animals ( $L$ ) in the population, and the rates of change among disease states. The first model portrays two disease states susceptible and infected with transmission occurring horizontally (Figure 3). This model represents the hypothesis that all transmission occurs by direct contact between infected and susceptible animals and that animals become infectious as soon as they are infected. In addition, we evaluated a model with horizontal transmission and a latency phase, which represents the hypothesis that transmission occurs horizontally but that animals become infectious during some period after initial infection (Figure 4). Alternatively, we composed a model that represents transmission between environmental residues deposited by infected animals (urine, feces) and susceptible ones (Figure 5). This model is based on the idea that the number of new infections per unit time per susceptible animal is directly proportionate to the mass of infectious material in the environment rather than the number of infected animals in the population. To obtain a simple model, we assumed that the rate of “uptake” of infectious material by susceptible animals was negligible relative to the rate of excretion by infected ones and the rate of loss of infectious material from the environment. This allowed us to ignore movement of infectious material from the environment into the susceptible compartment. Finally, our most detailed model portrayed a combination of horizontal and vertical transmission (Figure 6).

#### *Model Selection Procedures*

We used numerical integration (Runge Kutta order 4) to solve systems of equations and to predict cumulative CWD deaths. Solutions were iterated with non-linear gradient search techniques to find maximum likelihood estimates of model parameters and initial conditions. We evaluated strength of evidence in the data for each model using Akaike’s information criterion adjusted for small samples ( $AIC_c$ ) and Akaike weights.

### **Findings Relevant to Aim 1: Describe Mechanisms of Transmission**

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

This work remains in progress.

#### *Investigations of Environmental Transmission*

This work remains in progress.

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

#### *Development of Techniques for Sampling Live Animals*

Tonsil biopsy is a useful tool for estimating CWD prevalence in non-hunted mule deer populations, and the techniques we developed are being used in at least four other field studies of CWD

epidemiology (WY, NM, WI, SD). Data from this study were published in July 2002 (Wolfe et al., 2002, *J. Wildl. Manage.* 66: 564–573).

### *Local Scale Study: Effects of Land Use on Prevalence*

Because hunters cannot access urban areas as readily as non-urban, nearly all of the tonsil-based samples were taken from urban locations to achieve sample size objectives (Table 1). Mean prevalence rates and 95% confidence intervals for all groupings of sex and land use effects are shown in Table 2. Urban and non-urban land use types exhibited comparable prevalence rates ( $p = 0.400$ ). Overall, male prevalence rates were nearly double that of females ( $p = 0.004$ ), however land use appears to affect male and female prevalence rates differently. Although female prevalence rates were virtually independent of land use ( $p = 0.938$ ), males in urban locations had higher prevalence rates than non-urban males ( $p = 0.045$ ). Within urban locations, male prevalence rates were 2.5 times higher than female rates ( $p \leq 0.001$ ), while in non-urban locations male and female prevalence rates were similar ( $p = 0.223$ ). Site-scale effects influenced the overall probability of infection, with substantially lower prevalence rates at the EP site relative to GVM and HT ( $p \leq 0.001$ ). Deer in EP non-urban areas contributed more to this effect than urban deer ( $p \leq 0.001$  vs.  $p = 0.107$ ), irrespective of sex.

In addition to these bivariate contrasts, models describing the probability of CWD infection based on sex, land use, and site effects allowed us to assess the strength of support in the data for these effects (Tables 3 through 7). Two key pieces of information can be used to assess the relative support in the data for the various models. First, the  $\Delta\text{AICc}$  value estimates the relative expected Kulback-Liebler (K-L) distance, a measure of the information that is lost when a model is used to approximate truth. Using this approach, as  $\Delta\text{AICc}$  increases the amount of information lost relative to the best approximating model (minimum  $\Delta\text{AICc}$  value) increases. A set of “rules of thumb” have been devised to interpret the  $\text{AICc}$  differences (Burnham and Anderson 2002); models with  $\Delta\text{AICc}$  between 0-2 have substantial empirical support,  $\Delta\text{AICc}$  values between 4-7 show considerably less support, and  $\Delta\text{AICc}$  values  $>10$  have essentially no support in the data.

A second useful piece of information in Tables 3-7 is the  $w_i$ , or “Akaike weights” (Burnham and Anderson 2002), also called “model probabilities, which are the set of normalized model likelihoods found in the tables. The Akaike weight is considered as the weight of evidence in favor of model  $i$  being the actual K-L best model given that one of the models in the candidate set must be the K-L best model for that set of models. The bigger the  $\Delta\text{AICc}$ , the smaller the  $w_i$ , and hence the less plausible is model  $i$  of being the actual K-L best model based on the design and sample size used. Three classes of covariates were considered in the models and are represented in Tables 3-7 by the following codes; “Sex” indicates the sex of each deer, “Use” indicates if the sample was collected from an urban or non-urban land use type, and “EP” and “GVM” indicate which study site (Estes Park (EP) or Glacier View Meadows (GVM)) the sample came from. Since site effects were coded as dummy variables it was not necessary to include the Horsetooth (HT) site in any of the models. When all possible models, based on these three classes of covariates, were fit to the entire sample (Table 3) of data, only the first four models have support in the data based on the rules of thumb previously mentioned. Each of these top models contains both a Sex effect and the EP site effect. The presence of Use and GVM effects in two of the four models is related more to the fact that these effects occur in models containing the Sex and EP covariates more than to the presence of any substantial contribution being made to the models by the Use and GVM effects. Models without both the Sex and EP effects have virtually no support in the data. We conclude that coupling the higher prevalence rates exhibited by males with the relatively low infection rates seen at the EP site yields the best approximating models for estimating the probability of infection when all the data are used. These results also suggest that land use has little influence on the probability of infection when all of the data are used to develop the models.

The strong sex effect leads us to consider separate sets of candidate models for each sex (Tables 4 and 5). The results suggest differences in the factors influencing the probability of infection for each sex.

Data on CWD infections in female mule deer only support models containing an EP effect (Table 4). The top four models, which possess 99.2% of the Akaike weight, are the only ones that should be considered as plausible. Although land use is included in two of the four competing models, it contributes little to the overall fit of the model and is likely only present because it is coupled with the EP effect. Models based on male mule deer data (Table 5) have greater support for a land use effect than did the female-based models, however the strength of the EP effect does not allow the use effect to be competitive on its own. Thus, while land use type apparently influences the probability of infection in male deer, its impacts on female infection rates are negligible. We postulate that differences in behavior act as determinants of the differential response to land use between the sexes.

Finally, to explicitly model the probability of infection in urban versus non-urban areas we developed separate sets of candidate models for each land use type (Tables 6 and 7). An interesting dichotomy is seen between each of these sets of models. In urban areas (Table 6) the Sex and EP effects that were in all of the competing models based on the entire data set (Table 3) also appear in the top models. However, the Sex effect appears to be the dominant variable in models based only on the urban data. Models without the Sex covariate have little support in the data and receive almost no weight of evidence as being best approximating models. The reverse of this is seen in the models fit to the non-urban data set (Table 7). In areas of non-urban land use the EP site effect appears to be the variable of primary importance, with the strength of evidence for a Sex effect as the dominant predictor diminished relative to models fit to the urban data set. There is virtually no support in the data for models without an EP effect in non-urban areas. In urban areas the sex of a deer appears to be more important in determining the probability of being CWD positive than the site where the deer is located. However, in non-urban locations the site effect (EP) seems to play the greatest role in determining the infection status of a deer. As with the differences observed in the primary determinants of the probability of infection in males versus females, there may exist behavioral differences between urban and non-urban deer that determine which covariates are more indicative of infection status.

These results suggest that landscape features influence CWD at multiple scales and that the scale and degree of influence differs among males and females. The potential for development to alter the demographic composition of the deer population and subsequent prevalence rates suggests that urban areas may act to increase infection rates in male deer. Among adult males, it appears as if urbanization positively influences CWD prevalence rates. Concentrating older males in urban locations where there is little to no harvest pressure and where supplemental feeding is available throughout the winter may play a role in increasing prevalence rates in these areas. Unfortunately, we do not have data that allow us to compare the age structure of deer in urban and non-urban areas. Females may respond differently than males, for a variety of reasons, to the human footprint. Sex effects were considered *a priori* based on recent findings (Miller 2003, *unpublished data*) and our results support the observation that males have higher prevalence rates. However, not only did this analysis support those findings, but also the differential response to human development by the two sexes, with urban areas appearing to increase CWD prevalence in male and having little or no effect on female prevalence rates.

#### *Landscape Scale Study: Spatial Heterogeneity of CWD Prevalence*

We are currently fitting spatially structured Bayesian hierarchical models, relating biotic and abiotic factors to CWD prevalence at the scale of the individual home range using BUGS (Bayesian inference using Gibbs sampling) software (Spiegelhalter et al. 2003). Preliminary modeling results (Figure 7) suggest that coupling the spatial structure of CWD with non-spatial covariates provides a better representation of the observed distribution of prevalence rates than either model structure alone. This is merely an initial step, our goals over the next year include; 1) Gaining further understanding of the relative roles played by the aetiology of the disease and the landscape covariates in structuring the observed heterogeneity in prevalence rates, 2) Determining how these relationships change as the scale of analysis is varied, 3) Searching for ways to include patterns of deer movement and

connectivity between sub-populations and landscape attributes in order to incorporate covariates that are more biologically relevant to CWD spatial dynamics.

#### *Evaluation of an Urban CWD Management Strategy*

Data from our December pilot trial indicate testing and culling mule deer appears to be a viable approach for managing CWD in Estes Park. Based on the success of the first round of pilot testing, the Colorado Division of Wildlife has committed to a 5-year management experiment to evaluate the efficacy of test and cull in lowering CWD prevalence in an urban mule deer population.

#### *Future Research*

We hope to address temporal variations in disease dynamics when the time-series of data extends far enough to allow for the application of the model including a time component. Currently, the time series of observations of prevalence is not long enough to provide us with confident estimates of the rates of change in this disease across the landscape. Consequently, incorporating a temporal dimension to our work will necessarily remain one of the final pathways we explore. At the very least, we have instituted a modeling framework that will allow us to incorporate the time dimension of disease dynamics when the data support such an approach.

While our findings will not allow us to determine causal factors driving the observed spatial variations in CWD prevalence, they will suggest landscape attributes, and possibly biological mechanisms, that play an important role in mediating the spread of CWD. As an example, we will be able to infer the scale at which human-based alteration of the landscape bears the greatest influence on the distribution of CWD heterogeneity. Additionally, predictions made from these models will allow wildlife managers to develop prescriptions reflecting the intensity of harvest and culling efforts that are needed within a specific area to reduce prevalence rates.

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

Model selection revealed that the environment only model had almost 9 times more support in the data than the next best model, which represented horizontal transmission (Figure 8). There was no support in the data for the combined environmental and horizontal model, or for the horizontal model with latency (Figure 8).

We used the parameter estimates for the environmental transmission to develop a simple model of dynamics of CWD in free-ranging populations (Figure 9). The model suggested that CWD could reach a steady state in infected populations (Figure 9) at levels of prevalence resembling those seen in local population within the epidemic area (13%). Equilibrium dynamics might explain the seemingly paradoxical observation that prevalence of CWD in the epidemic area has remained virtually constant over the last 8 years.

Culling is widely used by wildlife management agencies as a technique for controlling prevalence of CWD. We derived steady state solutions for the number of infected and susceptible animals in the population and used those solutions to examine the ability of culling to eliminate CWD from a closed population (Figure 10) by manipulating the natural death rate in these steady state solutions. Analyses suggest that elevating the natural mortality rate with human hunting or culling can eliminate the disease (Figure 10). This conclusion is not particularly sensitive to the residence time of infectious material in the environment, even though the steady state in the absence of culling is very sensitive to that residence time (Figure 10). Simulations suggest that although eradication is theoretically feasible, sustained effort will be required to eliminate the disease, even from a closed population (Figure 11).

These results offer two fundamentally new insights. There is strong evidence in data that CWD may be transmitted from the environment to susceptible animals and that a steady state in prevalence can emerge from these dynamics. Because that steady state is controlled in part by the non-CWD mortality rate, our results suggest that widely used techniques for controlling the disease, in particular non-selective culling, can eradicate infected animals if applied consistently and for a sufficient period of time.

## References

- Bernardinelli, L., C. Pascutto, N.G. Best, W.R. Gilks. 1997. Disease mapping with errors in covariates. *Statistics in Medicine*. 16:741-752
- Besag, J. 1974. Spatial interaction and the statistical analysis of lattice systems (with discussion). *Journal of the Royal Statistical Society B*. 36:192-236.
- Besag, J., J. York, and A. Mollié. 1991. Bayesian image restoration with two applications in spatial statistics. *Annals of the Institute of Statistical Mathematics*. 43:1-59.
- Besag, J. and Kooperberg, C.L. (1995). On conditional and intrinsic autoregressions. *Biometrika*, 82, 733-746.
- Burnham, K.P., and D.R. Anderson. 1998. *Model Selection and inference: a practical information-theoretic approach*. Springer, New York, New York.
- Conner, M.E., Colorado Division of Wildlife. Preliminary Research Findings. March, 2002
- Cressie, N.A.C., 1993. *Statistics for spatial data- revised edition*. John Wiley & Sons, Inc. New York, New York. 900 pages.
- Geman, S. and D. Geman., 1984. Stochastic relaxation, Gibbs distributions and the Bayesian restoration of images. *IEEE Transactions on Pattern Analysis and Machine Intelligence*. 6: 721-741.
- Hurvich, C.M., and C-L. Tsai., 1989. Regression and time series model selection in small samples. *Biometrika* 76, 297-307
- Künsch, H.R. 1986. Intrinsic autoregressions and related models on the two-dimensional lattice. *Biometrika*. 74:517-524.
- Miller, M.W., E.S. Williams, C.W. McCarty, T.R. Sparker, 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.
- Mollie, A. (1996). Bayesian mapping of disease. In *Markov Chain Monte Carlo in Practice*. W.R. Gilks, S. Richardson and D.J. Spiegelhalter (eds.), New York: Chapman & Hall, pp. 359-379.
- Spiegelhalter, D.J., A. Thomas, and N. G. Best. 2003. BUGS: Bayesian inference Using Gibbs Sampling, Version 1.4
- Theobald, D.M., D. Schrupp, and L. O'Brien. 2001. Assessing risk of habitat loss due to private land development in Colorado. Final report for Cooperative Agreement No. 00HQAG0010, USGS-BRD Gap Analysis Program. 29 June. 62 pages.
- Williams, E.S., and S. Young. 1992. Spongiform encephalopathies of Cervidae. *Scientific and Technical Review Office of International Epizootis* 11: 551-567.
- Wolfe, L.L., M.M. Conner, T.H. Baker, V.J. Dreitz, K.P. Burnham, E.S. Williams, N.T. Hobbs, 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.

## Contributions

To date, we have developed a reliable, non-lethal method for detecting chronic wasting disease in mule deer. This represents a significant breakthrough in improving the ability to monitor prevalence of the disease over time and space. All previous methods for detecting the disease required killing animals. Our approach has been incorporated into other studies of CWD epidemiology, both locally and elsewhere.

We are the first group in the world to estimate transmission rate of chronic wasting disease in a captive population and the first to apply those estimates to understanding dynamics of free ranging animals.

We have provided the first evidence based on population modeling that widely used practices for eradicating chronic wasting disease can eliminate the disease in a closed population without eliminating the population.

### **Outreach Activities**

Miller participated in several public and interagency management meetings, providing updates on CWD epidemiology and management efforts in Colorado. He also participated in interviews for both local and national media, as well as a variety of public meetings on CWD epidemiology and management. Miller continued serving as an *ex officio* member of the Colorado Governor's Task Force on Chronic Wasting Disease, and as a member of the Regional CWD Management Work Group. Miller organized and moderated a special session on CWD at the Wildlife Disease Association's Annual Conference in Arcata, CA; the session highlighted several ongoing studies supported by this grant. Miller also presented overviews of CWD surveillance approaches at CWD symposia held in Denver and Edmonton, Alberta, presented a poster at the International TSE Conference in Edinburgh, and participated in a CWD Surveillance Workshop in Madison, WI.

Williams participated in many outreach activities during the past year including serving on the US Food and Drug Administration TSE advisory committee, as a consultant to the Institutes of Medicine committee on assessing prion science, and on a national Task Force to develop a CWD management plan with the USDA and the US Department of the Interior. She was a member of a panel convened to evaluate the CWD program in Wisconsin. She gave numerous presentations in scientific meetings and symposium including the International TSE Conference in Edinburgh, CWD symposia in Denver, Colorado and Edmonton, Alberta, and at a joint session of the United States Animal Health Association and the American Association of Veterinary Laboratory Diagnosticians. She participated in numerous interviews for local and national radio and print media.

Wolfe participated in several public meetings, providing updates on development and application of live animal tests for CWD. She presented papers on tonsil biopsy and its applications at the Wildlife Disease Association's Annual Conference in Arcata, CA, the national CWD symposium in Denver, the Rocky Mountain National Park research Conference, and the Colorado Chapter of the Wildlife Society meeting in Fort Collins; she also presented a poster at the International TSE Conference in Edinburgh. Wolfe also trained several wildlife veterinarians and biologists in tonsil biopsy techniques.

Farnsworth's contributions in the past year included:

Presentation: Estimating the relationship between chronic wasting disease and mule deer density across Northern Colorado. Wildlife Disease Association. Humboldt, California. July 2002.

Presentation: A comparison of three interpolation methods for estimating mule deer density. Ecological Society of America. Tucson, Arizona. August 2002.

Presentation: Mapping Chronic Wasting Disease in Northern Colorado mule deer. Wildlife Society, Colorado Chapter Meetings. Fort Collins, Colorado. January 2003.

Hobbs' outreach included two presentations this year:

Hobbs, N. T. Dynamics of chronic wasting disease in Colorado mule deer populations. Institute of Medicine, The National Academies, Washington D. C., 03/19/03.

Hobbs, N. T. Dynamics of chronic wasting disease: understanding transmission and prevalence in an urbanizing landscape. Institute of Ecosystem Studies, Millbrook, NY, 03/21/03.

### **Training and Education**

We are supervising the research of a PhD student and a master's student. We are providing training to a post-doc. Elizabeth Williams uses material from our project in the classes and the lectures she teaches at the University of Wyoming in both graduate and undergraduate seminars (Emerging Issues in Agriculture, Environmental Policy).

Hobbs used examples from the CWD project in teaching a graduate course in Systems Ecology. The project is one of the major research areas in an IGERT Project at Colorado State University, the Program in Mathematics, Ecology, and Statistics.

### **Publications**

#### **Journal Publications:**

Miller, M. W., and E. S. Williams. 2002. Detecting PrP<sup>CWD</sup> in mule deer by immunohistochemistry of lymphoid tissues. *Veterinary Record*: 151:610–612.

Williams, E. S., and M. W. Miller. 2002. Chronic wasting disease in deer and elk in North America. *Revue scientifique et technique Office international des Épizooties* 21: 305-316.

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

Williams, E. S. 2002. The transmissible spongiform encephalopathies: Disease risks for North America. *The Veterinary Clinics of North America Food Animal Practice* 18: 461-473.

Williams, E. S. 2003. Scrapie and chronic wasting disease. *Clinical Laboratory Medicine* 23: 139-159.

Williams, E. S., and M. W. Miller. 2003. Transmissible spongiform encephalopathies in nondomestic animals: Origins, transmission and risk factors. *Revue scientifique et technique Office international des Épizooties* 22 : in press.

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.