

RESEARCH PLAN

Chronic wasting disease (CWD) of the deer family is a transmissible spongiform encephalopathy, a member of a group of infectious diseases affecting animals and people known as prion diseases (Laplanche et al., 1999). 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 (PrP^{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 and southeastern Wyoming, where an epidemic of CWD has been ongoing for at least two decades (Williams and Young, 1992; Miller et al., 2000). In some areas within this region, as many as 15% of sampled mule deer (*Odocoileus hemionus*) are infected with CWD (Miller et al., 2000). 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 (World Health Organization, 2000).

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 to 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. Here, we propose studies to expand understanding CWD transmission mechanisms and to develop predictive models of disease dynamics. We focus on three specific aims:

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

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

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

To meet these objectives, we propose 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. We will use 15 years of data on CWD prevalence in selected mule deer populations to choose best approximating models of disease dynamics. We will then use these models to project likely trajectories of the disease in the face of dramatic anthropogenic environmental change.

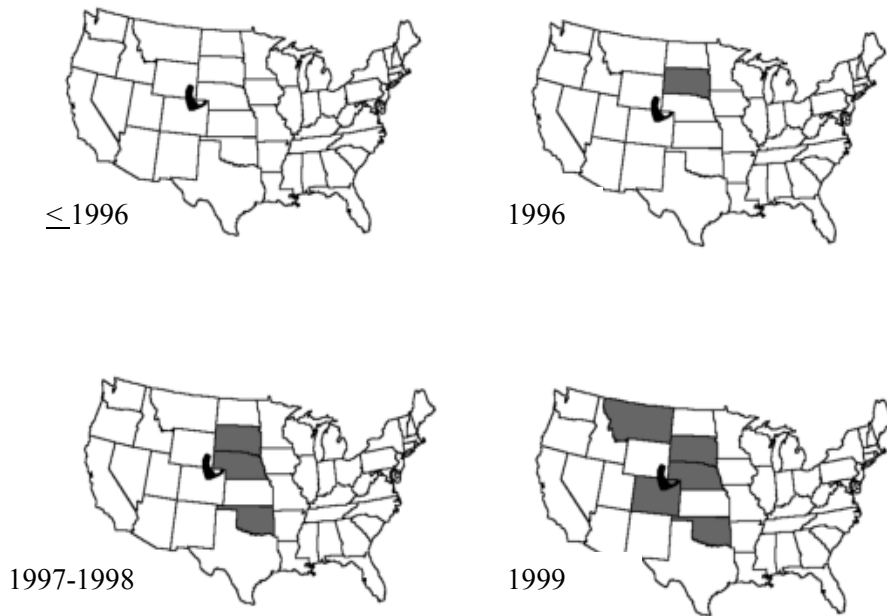
BACKGROUND AND SIGNIFICANCE

History

The emergence of bovine spongiform encephalopathy and the associated variant Creutzfeldt-Jakob disease (Will et al., 1996) that has affected >50 humans in Great Britain and France has motivated international concern about threats to human food supplies posed by transmissible spongiform encephalopathies (TSEs). The relationship between bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease was convincingly demonstrated by epidemiologic, pathologic, and molecular means (Collinge et al., 1996; Will et al., 1996; Bruce et al., 1997), and by use of transgenic mice (Hill et al., 1997; Scott et al., 1999).

Chronic wasting disease (Williams and Young, 1980, 1982), a unique TSE of captive and free-ranging deer (*Odocoileus* spp.) and elk (*Cervus elaphus nelsoni*), occurs only in North

Figure 1. Known distribution of CWD in the United States over time. The solid black area indicates the area of CWD in free-ranging cervids. The shaded areas show states where CWD has been found in privately owned, captive herds of deer and elk.



America. CWD in free-ranging cervids is endemic in northeastern Colorado and southeastern Wyoming (Spraker et al., 1997; Miller et al., 1998, 2000). However, within the last four years CWD has been recognized in privately owned elk in five states and one Canadian province, greatly expanding the known geographic range of the disease (Fig. 1). Wildlife management agencies and the public they serve have voiced strong concern about the potential for continental spread of CWD in commerce with possible exposure of native deer and elk across North America. Thus, CWD constitutes a major threat to native and captive wildlife (Williams and Young, 1992; Thorne and Williams, 2000; Williams et al., 2000). In addition, deer and elk with CWD are sympatric with range cattle in Wyoming and Colorado, and it follows that cattle are currently and historically potentially exposed to the prion causing CWD.

The relationships between CWD and other TSEs of domestic animals (e.g., scrapie, bovine spongiform encephalopathy, transmissible mink encephalopathy) and man (e.g., classic Creutzfeldt-Jakob disease) are not clear (Bartz et al., 1998; Raymond et al., in review); in particular, there is some speculation that CWD originated in deer exposed to scrapie-infected sheep (Spraker et al., 1997). Inclusion of CWD among the prion diseases is based on: 1) presence of spongiform lesions in brain (Williams and Young, 1980, 1982, 1993); 2) typical electron microscopic findings (Guiroy et al., 1993, 1994); 3) scrapie associated fibrils in brain (Spraker et al., 1997) and spleen (Merz and Williams, unpublished data); 4) amyloid plaques in brain (Bahmanyar et al., 1985; Guiroy, 1990; Guiroy et al., 1991b; Liberski et al., 1993; Williams and Young, 1993); 5) accumulations of immunohistochemically detectible PrP^{res} in brain and lymphoid tissue (Guiroy, 1990; Guiroy et al., 1991a, 1991b; Spraker et al., 1997; Sigurdson et al., 1999); and 6) detection of PrP^{res} on Western blots (Spraker et al., 1997; O'Rourke et al., 1999). PrP^{res} is the proteinase resistant abnormal isoform of the normal cellular glycoprotein (PrP^c) that accumulates in the TSEs and is thought to constitute the etiologic agent of the TSEs (Pruisiner, 1991, 1994, 1999).

CWD was first recognized as a clinical syndrome by biologists working with captive mule deer in Colorado in the late 1960s. The cause of the wasting condition in 3-5 year old deer was not understood until 1977 when CWD was recognized to be a spongiform encephalopathy (Williams and Young, 1980). Shortly thereafter, CWD was diagnosed in mule deer at the Wyoming Game and Fish (WGF) Department's Sybille Wildlife Research and Conservation Education Unit (Sybille). These facilities had exchanged animals for a number of years. A similar spongiform encephalopathy soon was recognized in captive Rocky Mountain elk (Williams and Young, 1982) which shared facilities with affected mule deer. A few cases of CWD were identified in free-ranging cervids in the early and mid 1980s, but it was not until 1993 that substantial numbers of cases in free-ranging mule deer were diagnosed in Colorado (Spraker et al., 1997) and fewer cases were diagnosed in Wyoming (Williams and Young, 1992; Williams and Thorne, unpublished data). CWD was retrospectively diagnosed in a white-tailed deer (*Odocoileus virginianus*) using immunohistochemistry in a 1991 case, the first recognition that this species was susceptible. Since 1977, natural CWD has been confirmed in approximately 250 free-ranging deer and elk within the endemic areas of Colorado and Wyoming (Miller et al., 2000). There are an estimated 60,000 free-ranging deer and 15,000 elk residing within the approximately 40,000 km² CWD endemic area (Miller et al., 2000).

Clinically, CWD is characterized by prolonged incubation periods of 1-3 years, protracted clinical course of weeks to months, and, invariably, death (Williams and Young, 1980, 1982, 1992; Miller et al., 1998). Consistent features include weight loss, behavioral changes, polydipsia, polyuria, and hypersalivation. Some animals, especially elk, may be ataxic and/or have muscle tremors. Clinical cases may occur at any time of the year, but in the wild the majority of cases are recognized during colder months. There is no apparent sex predilection. Chronic wasting disease is unusual among the TSEs in the high incidence of affected animals within captive populations; > 80% of mule deer in the Colorado facilities for ≥ 2 years developed CWD (Williams and Young, 1980). Similar prevalences have been observed in other herds of mule deer and white-tailed deer (Miller and Williams, unpublished data). These observations suggest a higher degree of susceptibility of cervids to CWD in comparison with susceptibility of sheep to scrapie and/or more efficient transmission among cervids.

In contrast to our relatively well-developed understanding of the pathology and clinical features of CWD, understanding of mechanisms of transmission remains primitive. Direct animal-

to-animal transmission of CWD has been implicated (Williams and Young, 1992; Miller et al., 1998). Although excreta are likely sources of infectious agent, the largest concentrations of PrP^{res} occur in portions of the brain, spinal cord, spleen, and lymph nodes of animals infected with scrapie or CWD (Farquhar et al., 1989; Ikegami et al., 1991; Race et al., 1992, 1998; Van Keulen et al., 1996; Roels et al., 1999; Spraker et al., in review; Williams and Miller, unpublished data); in sheep, PrP^{res} also has been demonstrated in placental tissues (Onodera et al., 1993, Race et al., 1998). These tissues are clearly infectious (Hoinville, 1996; Race et al., 1998; Sigurdson et al., 1999; Williams and Miller, unpublished data), but there is no obvious mechanism for release of PrP^{res} from the tissues of a live animal in high concentrations, save at parturition. Periparturient transmission of scrapie is apparently insufficient to sustain epidemics (Woolhouse et al., 1998, 1999; Matthews et al., 1999); similarly, CWD epidemics probably cannot be sustained by maternal transmission alone (Miller et al., 1999, 2000; Gross and Miller, in review).

In natural range settings, decomposing carcasses of scrapie- or CWD-infected animals could serve as an additional source of infection. Various combinations of scavenging and decomposition could play a role in releasing large quantities of PrP^{res} into the environment after an infected animal dies. Susceptible animals foraging near such sites subsequently could be infected by ingesting contaminated forage, soil, or water. Scavenging invertebrates actively exploit deer carcasses in CWD-endemic areas, and could be particularly instrumental in liberating PrP^{res} from skull-encased brain tissue. The recent demonstration of infectivity in maggots that had fed on scrapie-infected hamster brain (Post et al., 1999) lends plausibility to this potential mechanism. In addition to simple mechanical transfer of PrP^{res} into the environment, digestive processing could potentially enhance infectivity (e.g., Caughey et al., 1997; Caughey, 1999). Moreover, local environmental contamination could be amplified if PrP^{res} propagates in invertebrate hosts (e.g., Wisniewski et al., 1996). It follows that necrophagous, omnivorous, or adventive invertebrates could contribute to TSE transmission, particularly under range conditions.

Significance

Chronic wasting disease poses a fundamental threat to members of the deer family throughout Western North America. Because these animals play important roles in a many ecosystems (Hobbs, 1996), the threat posed by CWD transcends populations and could cause lasting harm to the functioning of many ecological processes throughout the region. Moreover, deer and elk contribute significantly to recreation-based economies in the Rocky Mountains and, consequently, reductions in the distribution and abundance of these animals will have serious economic impacts. Finally although direct threats of this disease to human health have not been established, they have not been ruled out. The World Health Organization recommends investment in improving understanding of CWD where it occurs (World Health Organization, 2000).

PRELIMINARY STUDIES

Here, we review preliminary studies our research team has undertaken in three areas: mechanisms of transmission and exposure, epidemiology and modeling, and impacts of land-use change on habitat for mule deer in the endemic area.

Mechanisms of Transmission

We have determined that CWD is laterally transmitted among deer and elk (Williams and Young, 1992; Miller et al., 1998; Miller and Williams, unpublished data). Both intra- and interspecific transmission can occur; CWD presumably arose in mule deer and subsequently crossed species lines to elk and white-tailed deer. Our observations suggest that CWD is probably transmitted among deer and elk by direct contact and/or environmental contamination. We have detected PrP^{res} in tonsil, lymph nodes, and nictitating membrane by immunohistochemistry in deer subclinically and/or clinically affected with CWD (Sigurdson et al., 1999; Williams and Miller, unpublished), providing evidence of potential routes for shedding the agent into the environment. The very early (42 days post inoculation) appearance of PrP^{res} in lymphoid tissue following exposure suggests the possibility of long term shedding of CWD agent during the prolonged incubation period.

Environmental contamination almost certainly accounts for some observations of CWD in deer and elk herds established for research at facilities in Colorado and Wyoming (Miller et al. 1998; Cook and Williams, unpublished; Miller, unpublished). Our work and work of others suggests that continuous shedding of infectious agent via alimentary tract excretions seems a plausible mechanism for scrapie and CWD transmission (Hoinville, 1996; Stringer et al., 1998; Woolhouse et al., 1998; Matthews et al., 1999; Miller et al., 1998, 1999, in review; Gross and Miller, in review). Even if shed at low concentrations, the remarkable environmental resistance of PrP^{res} could allow for build-up of infectious agent in contaminated environments. Such processes can measurably influence epidemic dynamics (Woolhouse et al., 1998), and may explain recurrence of scrapie and CWD cases in facilities that had previously housed infected animals (Greig, 1940;

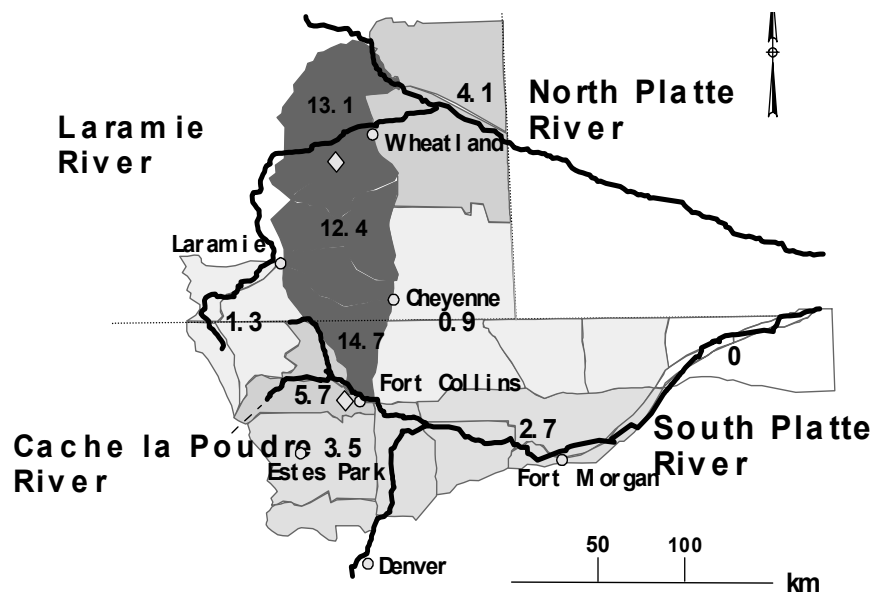


Figure 2. Prevalence of chronic wasting disease in Game Management Units along the Front Range in Northeastern Colorado. Numbers within shaded areas give % prevalence.

Sigurdsson, 1954; Pálsson, 1979; Sigurdarson, 1991; Williams and Young, 1992; Miller et al., 1998, and unpublished data).

Epidemiology and Modeling

Since 1983, we have been surveying selected populations of mule deer, white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) for presence of CWD using histopathology, supplemented more recently with immunohistochemistry (IHC) (Miller et al., in press; Williams, unpublished). Our study area focuses on a 38,137 km² polygon in southeastern Wyoming and northeastern Colorado where CWD is endemic in free-ranging cervids (Fig. 2) About 62,000 deer (mostly mule deer; *O. hemionus*) and 13,200 elk are distributed among numerous resident subpopulations. (Colorado Division of Wildlife [CDOW], unpubl. data; Wyoming Game and Fish Department [WGFD], unpubl. data).

For epidemiological investigations, we have collected samples from management units within and outside CWD-endemic portions of both Colorado and Wyoming (Fig. 2.) Management units (MUs) have been established by respective state wildlife management agencies to aid in population management and law enforcement. Boundaries are typically established with more regard for simplicity in recognition and description than for biological relevance. Consequently, we used MUs here only to provide an initial sketch of the approximate geographic origins of clinical suspects and harvested animals in order to assess spatial features of CWD epidemiology.

Between 1996 and 1998, we collected portions of medulla oblongata from 2,726 mule deer, 341 white-tailed deer, and 929 elk harvested, randomly culled, or road-killed in 29 MUs (Fig. 2) within or adjacent to the endemic foci identified via targeted surveillance. We also sample 757 deer and 760 elk from other MUs throughout Colorado and Wyoming (Miller et al., 2000). As applied here, IHC appeared to be both sensitive and specific in detecting CWD: 104 deer with histological lesions of spongiform encephalopathy tested IHC-positive (lower 95% CI bound = 0.965), and all 757 deer and 760 elk from outside known endemic MUs were IHC-negative (lower 95% CI bounds \square 0.995).

Our surveys revealed that CWD prevalence varied among the three cervid species residing in the endemic area. Overall IHC-based prevalence (prevalence; 95% confidence interval) in mule deer (4.9%; 4.1 to 5.7%) was somewhat higher than in white-tailed deer (2.1%; 0.5 to 3.4%), and much higher than in elk (0.5%; 0.001 to 1%). Only about 53% of IHC-positive mule deer and 50% of IHC-positive white-tailed deer had lesions of spongiform encephalopathy; none of the IHC-positive elk showed spongiform encephalopathy (Miller et al., in press).

Within species, CWD prevalence varied widely among biologically- or geographically-segregated subpopulations. For mule deer, estimated mean prevalence ranged from 0 to 14.7% among MU aggregates (Fig. 2). This variation in prevalence tended to follow biologically-relevant spatial patterns: lower elevation foothills subpopulations

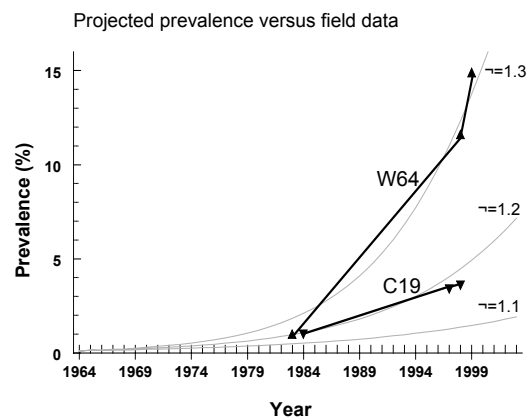


Figure 3. Projections of preliminary epidemic model of CWD (light lines) overlaid on field data on prevalence (dark lines) for two game management units (W64, C19). The β terms are transmission coefficients (number of infectious contacts per infected individual per year)

at the core of the endemic area tended to be most severely affected, with prevalence declining to varying degrees in all directions (Fig. 2). Similar but weaker patterns were evident among white-tailed deer and elk subpopulations.

Preliminary models of disease dynamics (Miller et al., 2000; Gross and Miller, in review) showed transmission rates are in the range of 1.2-1.3 infectious contacts per infected individual per year produced plausible epidemic dynamics (Fig. 3). Maternal transmission alone was not sufficient to sustain the epidemic, but did reduce the level of horizontal transmission necessary to sustain epidemics.

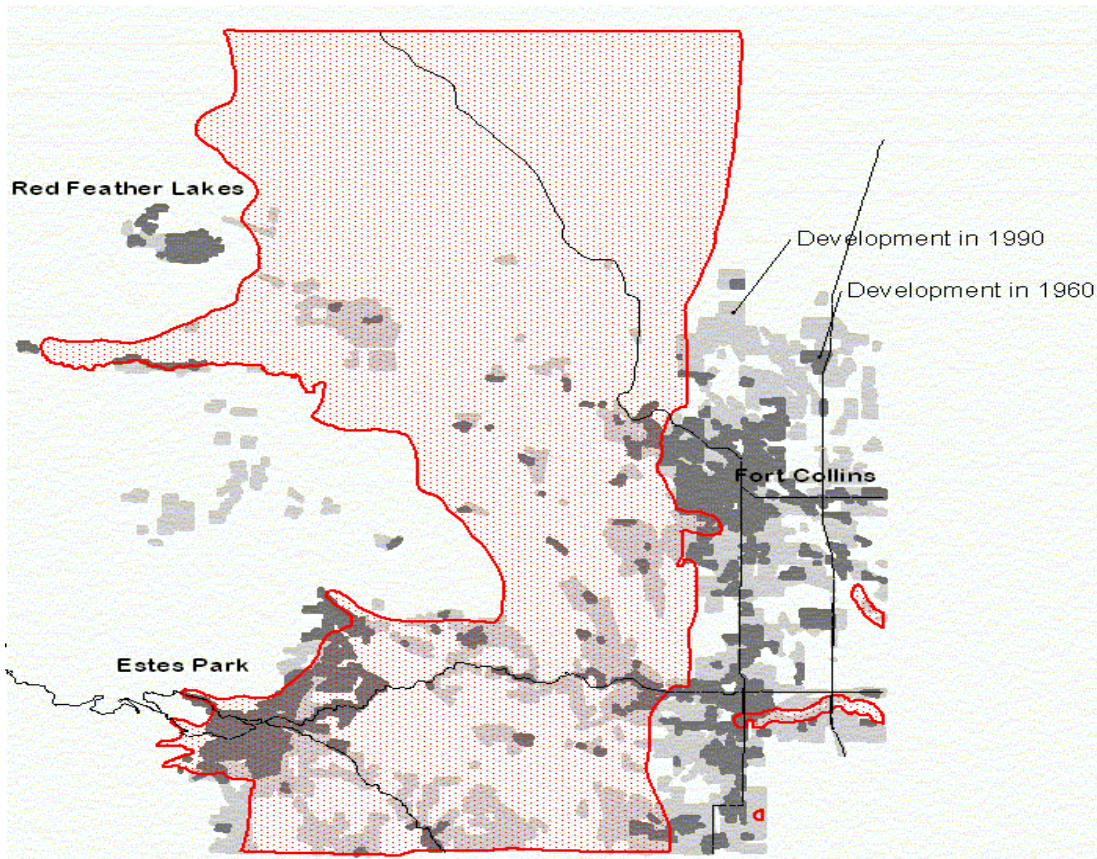


Figure 4. Changes in developed area within mule deer winter range in Larimer County Colorado during 1960-1990. The large polygon shows mule deer winter range. Dark shaded areas show development in 1960. Light shaded areas show expansion of development during 1960-1990.

Land Use Change

The area impacted by CWD also contains one of the rapidly growing human populations in the region. Economic trends favoring service and information based economies coupled with the aging of the baby boomers have enhanced mobility of the human population, and this mobility has led to rapid immigration to many areas of the west, particularly the northern Front Range of Colorado (Fig. 4) (Reibsame et al., 1996).

As an example, Larimer County is one main political units within the epidemic area. During 1990 to 1998 the county sustained population growth rates exceeding 3% annually. The area is projected to have over 342,000 residents by 2020. This is the equivalent of growing by an entire city of the size Fort Collins in two decades. There are about 636,400 acres of winter range for mule

deer in Larimer County. In 1960, about 5.3% of this was developed with housing densities >1 house per 40 acres. By 1990 the developed area of winter range has almost tripled, expanding to 17.6%. These trends have continued apace during the 1990s.

EXPERIMENTAL DESIGN AND METHODS

Overview

We will use a simple, base model of disease dynamics for organizing laboratory and field studies and as a point of departure for formulating a suite of alternative models to be tested against field data (Fig. 5). Alternative models represent formal statements of competing hypotheses on disease dynamics. Rather than testing hypotheses by conventional statistical methods, we will determine which model formulations have the greatest support in the data using approaches based on likelihood (Royall 1997) and model selection (Burnham and Anderson, 1998). Hypotheses with the strongest evidence will be represented by parameters and state variables remaining in models following structured confrontations between models and data (Burnham and Anderson 1998).

Our base model represents dynamics of CWD in a closed population as transitions among three states -- animals susceptible, exposed (i.e., latent) and infected (Fig. 5). Infected animals contribute to an external variable, which we will call environmental residue, consisting of urine, feces, and carcasses. Our experiences managing CWD in research facilities (Williams and Young, 1992; Miller et al., 1998, and unpublished data) strongly implicate these residues as potential routes of transmission. Upper limits on rates of transition from exposed to infected animals can be estimated from average preclinical periods in animals that eventually develop CWD, about 2 years (Miller and Williams, unpublished), but transmission parameters (i.e., β_l , β_m) can only be approximated based on current knowledge (Miller et al., in press). Unlike many models of this general type, we do not assume steady state because vital processes in the population occur more rapidly than incubation and transmission.

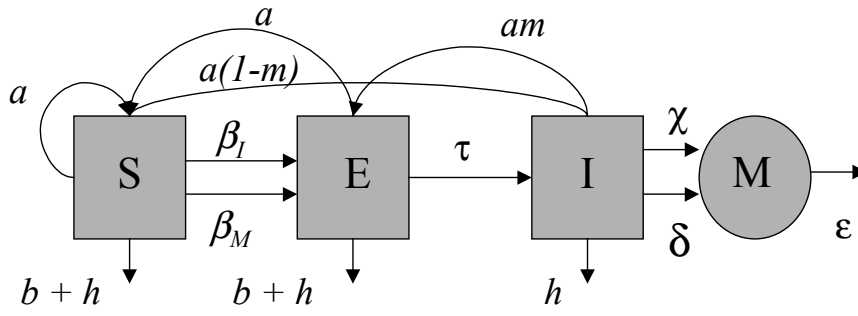
This model can be expanded by adding stage structure in the vector \mathbf{v} ,

$$\mathbf{v} = [S_f, S_b, S_d, E_f, E_b, E_d, I_f, I_b, I_d, M] \quad (1)$$

where S,E, I, and M are as defined above and subscripts reference age classes, fawns (*f*), and adult and yearling females (*d*) and adult and yearling males (*b*). Age classes beyond year 1 are usually combined in such models because field observations needed to estimate values for vital rates cannot distinguish between adults of different ages. However, this is usually not a problem because survival rates are very similar among animals older than 1 year (Bartmann, 1984), thereby justifying stage rather than age structure. A global model containing maximum detail can be represented as

$$\frac{\partial}{\partial t} \mathbf{v} = \mathbf{A}\mathbf{v} + \mathbf{f}(\mathbf{v}) + \mathbf{C}\mathbf{V}^2\mathbf{v} \quad (2)$$

where the matrix \mathbf{A} contains coefficients for births, natural deaths, hunting deaths, transmission, incubation, and dispersal. Coefficients controlling recruitment and survival can be set as constant



$$\frac{dS}{dt} = aS + aE + (1-m)aI - (b+h)S - \frac{\beta_I SI}{N} - \frac{\beta_M SM}{N}$$

$$\frac{dE}{dt} = \frac{\beta_I SI}{N} + \frac{\beta_M SM}{N} + maI - (b+h)E - \tau E$$

$$\frac{dI}{dt} = \tau E - hI - \delta I$$

$$\frac{dM}{dt} = I(\chi + \delta) - \epsilon M$$

S = density of susceptible individuals
 E = density exposed (latent) individuals
 I = density of infected individuals
 M = mass of infectious residue in environment
 β_I = infections per infectious animal per time
 β_m = infections per gram infectious material per time
 a = birth rate
 m = probability of maternal transmission
 b = rate of natural mortality additive to harvest
 h = harvest rate
 χ = urine, fecal excretion rate
 δ = mortality rate of infected animals (and individual to mass conversion)
 τ = incubation rate

Figure 5. Base model for organizing research and developing alternative, competing models of transmission of CWD.

per-capita rates, or can include linear or non-linear terms expressing effects of density dependence and influences of weather. The nonlinear vector f includes the interaction between susceptible animals and the sources of exposure. The final term represents spatial dynamics as a diffusion process. Although this notation provides a very compact expression, it should be understood that it represents a diverse spectrum of many simpler models obtainable by setting coefficients to 0.

Laboratory investigations will focus on understanding the mechanisms influencing transmission parameters m, β_I , and β_m (**Aim 1**). In particular, we will examine dose responses and will develop quantitative relationships between intensity of exposure and likelihood of infection by contact with infected individuals and by contact with environmental residues. We will determine if transmission can occur vertically, between mother and fetus. Field studies will provide data for maximum likelihood estimates of model parameters (**Aim 2**), and for selecting the best approximating model from the full suite of alternatives (**Aim 3**). We will also use field data to reveal the role of land use change in explaining current patterns of spatial variation in the disease. We will use best approximating models to project likely patterns of spread and efficacy of strategies for containment (**Aim 3**). Thus, the essence of our approach is to combine bottom-up, mechanistic studies of individuals in the laboratory with top-down phenomenological studies of populations in the field to arrive predictive models of temporal and spatial dynamics of the disease.

Laboratory Studies of Mechanisms of Transmission

Aim 1: Describe mechanisms of CWD transmission between infected and susceptible individuals and determine if environmental sources of infectious PrP^{CWD} can contribute to disease transmission.

Rationale

Although the existence of CWD in free-ranging populations of cervids has been established for nearly two decades, mechanisms of transmission remain poorly understood. An absence of understanding of the process of transmission hampers our ability to accurately understand and predict the dynamics of the disease. We propose laboratory studies to enhance understanding of transmission and in so doing, to support our efforts to model the disease in free-ranging populations.

Investigations of Shedding of PrP^{CWD} and Vertical Transmission

Hypothesis. The purposes of this investigation are to determine whether mule deer shed infectious doses of PrP^{CWD} in saliva, feces, urine, and/or reproductive tissues and fluids, and to characterize the timing and intensity of such shedding in order to refine understanding of transmission and improve parameterization of epidemic models.

Experimental Plan. Ten three-month-old, hand-raised female mule deer will be inoculated orally with a single oral dose of about 1g brain homogenate from CWD-infected mule deer; infectivity of this homogenate already has been demonstrated (Sigurdson et al., 1999; Williams and Miller, unpublished data). We will collect blood, saliva, urine, and feces from each deer every 6 months. In addition, all surviving does will be bred at about 15, 27, and 39 months post inoculation, and placental tissues and fluids collected at parturition. Upon development of clinical CWD, will be euthanized and subject to confirmational diagnostic examinations.

Fluid, excreta, and tissue extracts will be examined via Western blot (Wb) (Race et al., 1998; Lee et al., 2000; Miller and Larsen, unpublished data) and capillary immunoelectrophoresis (CIE) (Schmerr et al. 1999, Schmerr and Williams unpubl. data) for evidence of PrP^{CWD}. In addition, groups (n = 10/group) of transgenic mice (Westaway 1996) expressing native mule deer PrP^{sen} (sequences per O'Rourke et al., 1999) will be inoculated intracerebrally (IC) (20 µl, 10% suspension of test tissue or fluid) and intraperitoneally (IP) (100 µl, same respective suspensions) (Telling et al., 1994; Bruce, 1996) with pooled subsamples of saliva, urine, feces, and placental tissues collected at respective time steps; use of both inoculation routes may improve prion detection. Mice inoculated IC and IP with the aforementioned brain homogenate will serve as positive controls. Inoculated mice will be sacrificed upon showing neurological signs and subject to confirmational diagnostic examinations.

Data from Wb, CIE, and inoculation studies will be used to determine the route(s) and timing of PrP^{CWD} shedding in CWD-infected mule deer, and to semiquantitatively compare concentrations of PrP^{CWD} among samples (e.g., Race et al., 1998; Schmerr et al., 1999; Lee et al., 2000). Infection rates and incubation periods in groups of inoculated mice also will be used to compare intensity of PrP^{CWD} shedding within and among respective sources. Experimental data will be scaled for model parameter estimations by comparing mean incubation periods in inoculated deer to mean incubation periods in naturally-infected deer cohorts (Miller unpublished data).

Expected Results. Based on previous studies, most orally inoculated deer will develop clinical CWD 18-30 mo post inoculation (Williams and Miller, unpubl. data). We anticipate detecting PrP^{CWD} (via both direct analyses and bioassays) in saliva and feces, and perhaps placental tissues, coincident with appearance of clinical signs, if not before. We further anticipate showing that concentrations of PrP^{CWD} in excreta increase with time throughout CWD's clinical course in mule deer.

Potential Limitations/Pitfalls. At the time of proposal submission, Wb and CIE assays have not been completely validated for use in detecting PrP^{CWD} in excreta; however, such studies are already underway and methods will likely be refined within the next 12 months. Similarly, transgenic mice expressing native mule deer PrP are currently in development, and should be available within the next 6-12 months for preliminary evaluations of susceptibility to CWD. In the unlikely event that transgenic mice prove to be inadequate hosts for PrP^{CWD} propagation, we will use hand-raised mule deer (n = 3/excreta × time combination) held in indoor isolation facilities to assess infectivity of saliva, urine, feces, and placental tissues from CWD-infected mule deer. Inoculation methods will be as described, but inoculum volumes will be increased to about 1 ml IC and 10 ml IP.

Studies of Environmental Transmission

Hypothesis. The purposes of this investigation are to determine if indirect transmission of CWD occurs via exposure to environmental sources of PrP^{CWD}, to gauge the relative intensity of indirect transmission from different sources, and to use the foregoing data in refining parameter estimates in epidemic models of CWD in mule deer.

Experimental Plan. We will compare occurrence, mean time to clinical onset, and mean infection intensity of CWD infections among groups of mule deer potentially exposed to PrP^{CWD} via direct and indirect natural sources of infection. Experimental exposure treatments will include: 1) housing with other naturally-infected deer; 2) housing in paddocks that had previously held CWD-infected deer; 3) housing in paddocks where carcasses from CWD-infected deer had decomposed naturally; or 4) housing in paddocks with no known exposure to CWD-infected animals. We will use a randomized complete block design, with three replicate paddocks per treatment and five experimental subject deer per replicate paddock. Weaned mule deer fawns will be captured and stocked into experimental paddocks using standard protocols (Miller et al., 1998).

Each subject deer will be evaluated at least monthly and subjectively scored (0 = not shown, 1 = subtle, 2 = obvious) for behavioral changes, loss of body condition, ataxia, and salivation or polydipsia. Subject deer with clinical scores 3 will be regarded as clinical CWD cases and euthanized. Because animal-to-animal transmission within groups could easily confound transmission from environmental sources, all subject deer in an individual paddock will be sacrificed and subjected to complete necropsy and tissue sampling when the first clinical case occurs among subject deer in that paddock. (Provided no clinical CWD cases occur in control paddocks, all subject deer in control paddocks will be sacrificed 12 months after the last group of exposed subject deer is sacrificed.)

Carcasses will be subjected to complete necropsy and tissue sampling. Brain and select lymphoid tissues will be examined by IHC and histopathology using established techniques (Williams and Young, 1993; Spraker et al., 1997; Sigurdson et al., 1999; Miller et al., 2000). We

will note distribution of PrP^{CWD} and microscopic lesions as determined by IHC and histopathology, and use these to score the chronological progression of CWD in each subject deer. Based on observations from ongoing studies of CWD pathogenesis in mule deer (Spraker et al., in review; Williams and Miller, unpublished data), scores will be assigned as follows: 0 = no evidence of PrP^{CWD} accumulation via IHC in either lymphatic or nervous tissue and no spongiform encephalopathy; 1 = PrP^{CWD} accumulation in tonsil and/or other lymphatic tissue but not in nervous tissue; 2 = PrP^{CWD} accumulation in both lymphatic tissue and nervous tissue; 3 = PrP^{CWD} accumulation in lymphatic and nervous tissue, and spongiform encephalopathy.

Our primary study objective is simply to document indirect transmission of CWD via exposure to contaminated environments. If CWD occurs in deer held in contaminated paddocks in the absence of carrier animals, then we will conclude that contaminated environments could be a source of CWD infection under natural conditions that should be considered in epidemic models and control programs. Beyond determining whether or not CWD transmission occurs under respective exposure sources, we will use two measures (times to initial clinical onset, group infection intensities) to compare the relative intensity of CWD exposure among experimental treatments. Influences of exposure source on times to initial clinical onset and infection intensities among treatment groups will be evaluated using Akaike's Information Criterion for small samples (Burnham and Anderson, 1998). Based on our results, epidemic model assumptions and parameter estimations will be adjusted to incorporate experimental findings.

Expected Results. We anticipate that mule deer exposed to CWD via either infected deer or contaminated environments will become infected, but that unexposed control deer will remain uninfected. Based on previous experimental data and observations, the minimum incubation period (inoculation to clinical onset) of CWD in mule deer is about 12-15 months after oral challenge, about 20-22 months after cohabitation with heavily infected groups of captive deer, and about 30-36 months after confinement in contaminated paddocks (Miller et al., 1999; Williams and Miller, unpublished data). Consequently, at least one subject deer in most exposed paddock groups will probably develop CWD within 24-36 months, regardless of exposure source. In general, we expect direct exposure to produce the most intense infections, followed respectively by exposure to carcass-contaminated and excreta-contaminated environments. We predict that most subject deer in most treatment paddocks will, at minimum, show lymphoid IHC staining typical of early preclinical CWD infections at the time of sacrifice. However, the overall magnitude of infection and relative infection intensities within and among treatment groups remain to be determined.

Potential Limitations/Pitfalls. Based on our previous observations and experiences with CWD in captive mule deer, it seems unlikely that indirect transmission will fail to occur at some level. Because these mechanisms of transmission have not been demonstrated previously, we recognize that there is some small chance that indirect transmission will not occur under the experimental conditions outlined here. It seems more likely that our results may tend to overestimate the magnitude of these processes, particularly when compared to free-ranging conditions, because our experimental conditions are relatively extreme. Sample sizes are limited by the size and number of paddocks available at the CDOW's FWRP, especially with respect to paddocks inhabited or contaminated by CWD-infected deer. The combination of small sample sizes and extreme exposure conditions may preclude our ability to demonstrate differences in the intensity of infections resulting from exposure to CWD from different sources.

Field Studies for Parameterizing and Selecting Models

Aim 2: To describe spatial and temporal variation in disease prevalence.

Rationale

Although preliminary efforts have provided a foundation for modeling temporal dynamics of CWD, initial estimates of model parameters remain coarse, and our ability to predict dynamics of CWD are correspondingly limited. We will extend preliminary data to allow reliable estimation of transmission rates and prediction of changes in prevalence over time. We will also parameterize spatial models. Preliminary studies have shown substantial spatial variation in CWD prevalence (Miller et al., 2000). We are interested in the extent to which anthropogenic effects on land cover influence this variation. It is clear that shifts in land-use within the epidemic area are a fundamentally important source of environmental change (Theobald et al., 1999). Conversion of traditional deer habitat into development can have different effects on the distribution of deer, effects that could potentially accelerate or retard the spread of the disease.

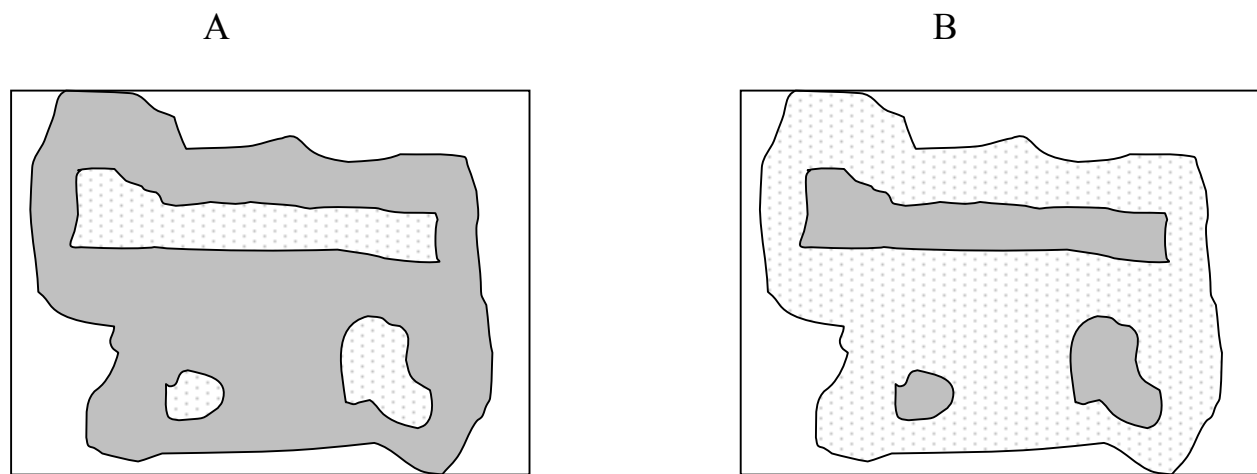


Figure 6. Illustration of habitat perforation (panel A) and fragmentation (panel B). In both cases, the dotted area represents development and the shaded area represents deer habitat. Areas of the landscape that are perforated increase population density without affecting animal movement. Habitats that are fragmented increase density and restrict movement among subpopulations.

Accelerating effects could result from land-use patterns that “perforate” deer habitat, thereby reducing habitat area and increasing densities without reducing movement of infected animals among populations (Fig. 6A). Habitat perforation can also reduce the ability to control deer abundance via hunting, further magnifying increases in density. Preliminary modeling efforts (Gross and Miller, in review) suggest that such increases are likely to increase the size of the infected pool, and could increase transmission to unaffected populations.

Conversely, retarding effects could result from land-use patterns that fragment habitat (Fig. 6B). That is, if patterns of development isolate subpopulations of deer, then it is plausible that transmission among sub-populations would be reduced (Hess, 1996), and that truly isolated, infected populations might decline to extinction.

The objective our field studies is to obtain data on temporal and spatial patterns of the disease needed to parameterize and select dynamic models for understanding and predicting the spread of CWD in time and space.

Study Area

Field studies will focus on 3 subpopulations of mule deer in Northeast Colorado, Bellvue/Masonville (GMU 19/20), Redfeather/Livermore (GMU 191) and Virginia Dale/Boxelder Creek (GMU 9) (Fig. 7A). These populations have prevalence of CWD ranging from 5 to 15% (Fig. 7B). The area used by these populations includes a broad range of land-uses, including urban and suburban, agriculture, and National Forest (Fig. 7C). The area has correspondingly broad range in human population density. Elevations range from 1800 m to over 4000 m, and this gradient gives rise to a broad spectrum of land-cover types (Fig. 5D).

Field Procedures

In all of these areas, deer populations are inventoried annually by the Colorado Division of Wildlife using helicopter counts of random 2.59 km² quadrants. They are also sampled for sex and age distribution during mid-winter helicopter classification surveys, and are monitored for adult and juvenile survival based on 40-80 radio-collared fawns and adults. Hunter harvest is estimated from telephone surveys. These observations provide a basis for estimating population density as well as vital rates in population models (Fig. 5). For most of the study area we have 9 years of these population data and will obtain an additional 4 years during the course of the proposed work.

To estimate CWD transmission rates, we will collect heads from all animals harvested by hunters in these study areas and will supplement harvest samples with randomly culled animals each year of the study where necessary to achieve target sample sizes of 200-400 samples within each subpopulation during each year (sample size necessary to estimate prevalence at $\pm 3\%$ of true values 95% of the time). We will cull additional animal each year to achieve broad coverage across all land-use types. Approximate locations for culling animals will stratified by land use category (National Forest, urban, suburban, agricultural) and culling effort will be allocated to assure 200-400 observations for each land-use category for each year of the study. We will attempt to collect animals in sex and age classes in approximate proportion to their occurrence in the population. For all deer sampled, we will record the location where the animal was harvested

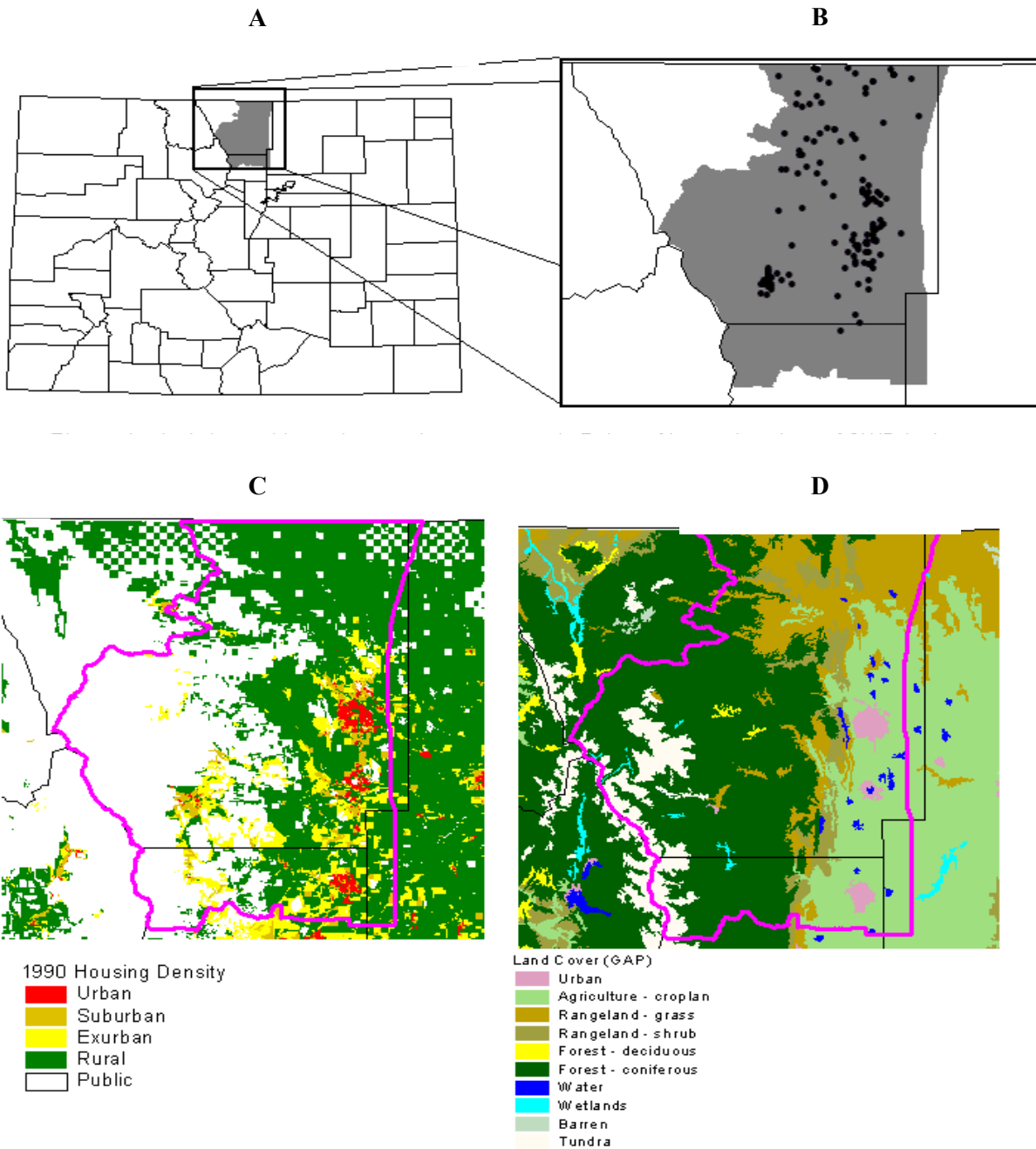


Figure 7. Study area for field investigations of prevalence of chronic wasting disease in mule deer populations. **A.** Locations of study areas in Colorado. **B.** Spatial distribution of observations of diseased animals. **C.** Housing density within the study area. **D.** Major land cover types within the study area.

or culled (UTM), its sex, and age. Brainstem (medulla oblongata at obex) and tonsil tissues will be collected and prepared using standard techniques (Miller et al., 2000).

We will use immunohistochemistry (IHC) to confirm CWD diagnoses. Briefly, formalin-fixed, paraffin-embedded tissues will be sectioned, processed and stained using minor modifications of techniques described previously (Miller et al., 1993; van Keulen et al., 1995). We will use mouse monoclonal antibody (MAb) against bovine PrP (F89/160.1.5; USDA/ARS, Pullman, Washington) (O'Rourke et al., 1998). Prior to immunostaining, sections will be immersed in 99% formic acid for 30 to 60 min followed by hydrated autoclaving for 10 to 20 min. Sections will be immunostained with diluted primary antibody for 0.5 to 1 hr at 37 C, a biotinylated anti-rabbit or anti-mouse secondary antibody as appropriate, an ABC reagent (Vectastain ABC Kit, Vector Laboratories) or alkaline phosphatase streptavidin conjugate, a substrate chromagen (fast red or AEC), and a hematoxylin counterstain. Positive and negative brain sections, normal rabbit or mouse serum, and PBS will be used as controls. We will also use histopathology of brain tissue with particular attention to the medulla oblongata at the obex (Williams and Young, 1993; Spraker et al., 1997) to further stage CWD progression. Tissues will be sectioned at 5-6 μm and stained with hematoxylin and eosin. Lesions will be compared to those described for CWD in mule deer, white-tailed deer, elk, and other natural TSEs of animals (Williams and Young, 1993; Hadlow, 1996; Spraker et al., 1997). Sampled deer will be classified as CWD-positive or negative; positive deer will be further categorized as early- (tonsil staining only), mid- (tonsil and brainstem staining), or late-stage (tonsil and brainstem staining, with spongiform encephalopathy) CWD cases (Sigurdson et al., 1999; Miller et al., 2000; Spraker et al., in review). Early and mid stage categories will be equated with exposed animals and late with infectious. (This categorization may be modified based on our laboratory studies.)

Expected Results

We are confident that we will be able to obtain over 10,000 observations of disease state, observations spatially dispersed throughout the epidemic area. These observations will provide a strong foundation for selecting models, as described in the next section.

Potential Limitations/Pitfalls

The large samples required to estimate prevalence necessitate some opportunism in sampling regimes. We recognize that the opportunities for observing disease state offered by hunter-killed animals could create spatial bias in our samples. However, we plan to remedy this problem by culling or darting and euthanizing animals in areas where hunter returns are underrepresented. We can similarly cope with biases in sex and age distribution of samples.

Model Selection and Inference

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

Rationale

The ability to understand and predict the spatial and temporal dynamics of CWD ultimately depends on assembling knowledge in a model of the operation of the disease in infected populations. As with all ecological processes, the challenge in building models of CWD is

choosing the appropriate level of abstraction---retaining parameters that are essential to describing the process while eliminating those that are nonessential. Until recently, this was a largely subjective choice. However contemporary developments in information-theoretics allow objective decisions on model detail, by using data to arbitrate the appropriate level of parsimony (Burnham and Anderson 1998). Here, we outline data analysis procedures that will permit us to select best approximating models on dynamics of CWD in mule deer.

Model Selection Procedures

We will use likelihood-based methods (Buckland et al., 1997; Burnham and Anderson, 1998) to quantify strength of evidence for various epidemic models for CWD (equation 2), to estimate parameters in the face of model uncertainty, and for evaluating factors (predictors) effecting spread of the disease. Although the details of these statistical approaches are spelled out in Burnham and Anderson (1998) they are relatively new, hence we offer a brief overview here.

The critical philosophy underlying these methods is that all models for "explaining data" in life sciences are simplified approximations to very complex truth. When there are competing models as approximations to truth we can use the Kullback-Leibler information discrepancy, $I(f, g)$ to measure information loss when model g is used to approximate truth f . This measure has a deep theoretical basis (see review in Burnham and Anderson, 1998). Skipping all the theory, we simply note that K-L, combined with maximum likelihood estimation (MLE), and finite samples, leads to Akaike's Information Criterion, AIC, and variations on it for model selection and extensions to multi-model inference. We give here only the basic technical ideas, even though the underlying philosophy is also important (Burnham and Anderson 1998).

There will be a set of R (parametric) models considered, denoted here simply as g_1, \dots, g_R . A partial list of these candidates is summarized in Table 1. Each model has parameters that must be estimated from the data; generically these are denoted as θ , or more specifically, as θ_r . The MLE is $\hat{\theta}_r$. Model r has K_r estimable parameters. Fitting the model to the data means estimating its parameters; maximum likelihood is used. Most of these parameters are structural, but an unknown variance (σ^2) is also a parameter and is included in r and K_r . The likelihood function $\mathcal{L}(\theta | data, g)$ is just the model $g=g(data|\theta)$ considered as a function of θ given the data. The maximized likelihood, $\mathcal{L}(\hat{\theta})$ occurs at the MLE of θ . Large sample AIC is

$$AIC = -2 \log \left(\mathcal{L}(\hat{\theta}) \right) + 2K \quad (3)$$

Table 1. Summary of candidate models derived from base model (Fig. 5) of dynamics of CWD in mule deer populations. A family of alternative models corresponding to different hypotheses on spatial and temporal dynamics results from adding or removing variables and expressions from the base model.

Hypothesis represented in change to model.	Modification of base model	A priori basis for modification
Maternal transmission does not affect population dynamics.	Set probability of maternal transmission (m) = 0.	Gross and Miller, submitted
Environmental residues do not affect transmission.	Remove state variable representing environmental	

Hypothesis represented in change to model.	Modification of base model	A priori basis for modification
Disease death rates do not exceed natural death rates.	contamination. Set rates of natural death + hunting deaths = rates of mortality from CWD	
Differences in sex and age affect transmission.	Add age and sex structure to model.	Miller et al. 2000
Mortality rates are density dependent.	Add linear or non-linear expressions for natural mortality by age class as a function of population density.	(White and Bartmann 1998)
Mortality rates respond to annual differences in winter weather.	Add linear or non-linear expressions for natural and disease-induced mortality as a function of winter weather.	(Bartmann, 1984)
Spatial variation CWD can be partially explained by spatial variation in land use and land cover.	Add diffusion term with map of resistance to movement derived from land-use, land-cover maps.	(Theobald et al. 1996, Theobald et al. 1997)

As with likelihood theory in general, a single AIC value is neither useful nor interpretable and is only defined up to a arbitrary additive constant. But when we have AIC for each model, hence AIC_r , then the differences $\Delta_r = AIC_r - \min(AIC)$ are useful and interpretable as to model selection uncertainty. Here, $\min(AIC)$ is the value of the smallest AIC_r value: $AIC_r = \min\{AIC_1, \dots, AIC_R\}$. The model corresponding to the $\min AIC$ is the single best model for the data at hand. Refinements to AIC exist, in particular AIC is a large sample result; a better version is

$$AIC = -2 \log \left(\mathcal{L}(\hat{\theta}) \right) + 2K + \left(\frac{2K(K+1)}{n-K-1} \right) \quad (4)$$

AIC is often presented in the scientific literature in an ad hoc manner, as if the term $2K$ is just a penalty for model dimension. Worse yet, perhaps, is that AIC is often given without reference to its fundamental link with Kullback-Leibler information. It is critical to realize that AIC and AIC_c are estimates of expected relative Kullback-Leibler distance and are useful in the analysis of real data in the noisy sciences. The Δ_r values are easy to interpret and allow a quick comparison and ranking of candidate models and are also useful in computing Akaike weights (below). As a rule of thumb models having $\Delta_r \leq 2$ have sufficient support---they should receive consideration in making inferences. Models having Δ_r within about 3-7 have considerably less support, while models with $\Delta_r > 10$ have essentially no support.

Akaike weights, w_r , are a very important aspect of multi-model inference and assessment of model selection uncertainty. They are based on the likelihood of a model given the data as

$$\mathcal{L}(g_r | data) = e^{\left(-\frac{1}{2}\Delta_r\right)} \quad (5)$$

It is convenient to normalize these model likelihoods so they are relative weights that sum to

$$w_r = \frac{e^{\left(-\frac{1}{2}\Delta_r\right)}}{\sum_{i=1}^R e^{\left(-\frac{1}{2}\Delta_r\right)}} \quad (6)$$

The relative likelihood of model r versus model j is w_r/w_j . Alternatively, these w_r may be thought of as probabilities (but they are not proper posterior probabilities such as Bayesian theory generates). Then we can interpret w_r as the estimated probability that model r is the K-L best model for the data at hand, given the set of models considered. Thus, the model selection uncertainty (given the set of R models) is provided by these Akaike weights.

Another use of these weights is for unconditional inference on a parameter in common over all models (called model averaging); this includes predictions that can be based on any of the models (extensions exist to the case when a parameter is not in common to all R models). Here, let $\hat{\theta}_r$ denote the MLE of scalar parameter θ based on model r . Let $\hat{var}(\hat{\theta}_r | g_r)$ be the usual estimated sampling variance of $\hat{\theta}_r$ conditional on model r . The model averaged estimate of θ is

$$\hat{\theta} = \sum_{r=1}^R w_r \hat{\theta}_r \quad (7)$$

It is often better to use this model averaged estimate rather than to simply use the estimate of θ that comes from the selected best model.

Variable selection is another aspect of model selection (i.e., the models involve predictor variables, x_j , as in regression) which we will use in our studies of environmental controls on spatial distribution of CWD. Variable selection is better thought of as an issue of finding the relative variable importance for each variable. The relative importance of variable x_j is simply the sum of the model weights w_r over the models in which x_j appears. This is another form of multi-model inference, and it is especially important for the objective of identifying important predictive factors. We will use variable importance as sums of Akaike weights to identify predictor importance.

Selecting Models Representing Temporal Dynamics

We will begin by assuming a closed population to focus on temporal dynamics (i.e., we will remove the diffusion term from equation 2). We will use data on population size, age and sex composition, and disease prevalence in the three study populations to estimate parameters in candidate models (Table 1) by confronting predictions of state variables with field observations of those states. Model predictions will be obtained numerically as follows.

Depending on which candidate model we consider, the system modeled can include four ordinary differential equations or a mixed system of partial differential equations (hyperbolic and/or parabolic) and ordinary differential equations. In all cases, the systems will be sufficiently complex that we will not be able to obtain analytic solutions. Instead, systems will be analyzed to determine the existence of propagating waves and the systems will be solved numerically. There

are a variety of methods that can be used to solve the system when we have ordinary differential equations. When we have a mixture of partial differential equations, we will use explicit schemes for the partial differential equations and an Euler's method for the ordinary differential equations so that the solution schemes will be compatible. Care will be taken to use the proper differencing techniques when the partial differential equations are hyperbolic or when the diffusion coefficients are small (Thomas, 1991a). When steep fronts appear (which are common in the spread of epidemics), higher order methods will be used (Thomas, 1991b).

Model parameters will be estimated using likelihood techniques (Edwards, 1992; Hilborn and Mangel, 1997; Clayton and Hills, 1998). We will examine data from preliminary field studies (Miller et al., in press) to choose appropriate error structures. Parameter estimates will be constrained based on results of laboratory studies. For example, if our experiments reveal that vertical transmission does not occur, the value representing such transmission will be constrained to = 0. Other routes of transmission will be constrained based on results of dose experiments to allow plausible values. Maximum likelihood estimates of constrained parameters will be obtained by non-linear optimization. A range of state variables and observations can be included in the likelihood function by scaling the squared difference between observations and predictions by the variance in the observations. Although it is reasonable to assume that most of the variation in field estimates of vital rates and disease prevalence can be attributed to observer error, we will parameterize models separately under assumptions of observer error and process error (Pascual and Kareiva, 1996; Hilborn and Mangel, 1997).

Models will be ranked using AIC_c and Akaike weights will be used to assess model selection uncertainty. Parameters in the best 3-5 candidate models will be averaged using these weights as described above. We will use variable selection to assess the relative importance of state variables and their associated parameters.

Selecting Models Representing Spatial Dynamics

We first provide an overview of our approach, and then give some details. We wish to examine environmental features that affect movement of the disease, particularly the influence of perforating, fragmenting, and compressing effects of development on the spatial configuration of deer habitat. We will use techniques similar to those used in ground water modeling and oil reservoir simulation, where numerical solutions to diffusion models are governed by input data on "porosity" or relative resistance to diffusive movement. These data are represented as "porosity" matrices. We will use non-linear search techniques to find values in the porosity matrices that provide the best fit between model predictions of simulated maps of disease prevalence and observations of prevalence derived from point data.

To simulate changes in spatial patterns, we will modify the best approximating temporal models (chosen following procedures described above) by replacing the laplacian term with matrices representing movement of susceptible and infected deer ($\mathbf{B}_1, \mathbf{B}_2$):

$$\frac{\partial}{\partial t} \mathbf{V} = \mathbf{A}\mathbf{V} + \mathbf{f}(\mathbf{V}) + (\mathbf{B}_1 \mathbf{V}_x)_x + (\mathbf{B}_2 \mathbf{V}_y)_y \quad (8)$$

\mathbf{B}_1 and \mathbf{B}_2 are diagonal matrices with $\beta_1^1, \dots, \beta_9^1, 0$ and $\beta_1^2, \dots, \beta_9^2, 0$ on the respective diagonals. The β terms represent relative resistance (or attraction) in particular directions; they are not constant but instead vary with spatial position on the landscape. The diffusive term associated with any particular equation (except for biomass of infective residues, M) will be of the form:

$$(\beta^1 u_x)_x + (\beta^2 u_y)_y \quad (9)$$

To solve these equations numerically, a grid will be placed over the region of interest with a control volume associated with each grid point. The diffusive term will be differentiated as

$$\frac{1}{\Delta x^2} \left[\beta_{j+1/2,k}^1 (u_{j+1,k} - u_{j,k}) - \beta_{j-1/2,k}^1 (u_{j,k} - u_{j-1,k}) \right] + \frac{1}{\Delta y^2} \left[\beta_{j,k+1/2}^2 (u_{j,k+1} - u_{j,k}) - \beta_{j,k-1/2}^2 (u_{j,k} - u_{j,k-1}) \right] \quad (10)$$

Note that the β 's are evaluated at the interfaces ($j+1/2$, $j-1/2$, $k+1/2$ and $k-1/2$) of the control regions. These values control the flow of animals across the appropriate boundaries of the control regions. We will use nonlinear optimization to find the value of β terms that give the best fit between model predictions and observations of the spatial distribution of infected deer over time. These best fit values will be used to create a "porosity" grid which expresses resistance to movement of the disease in a spatially explicit fashion.

Observations of deer distribution will be related to the porosity grid using kernel density estimators. Assume a map exists showing the location of all the healthy and infected deer in some bounded region, A . One way to summarize the spatial distribution of the deer is to estimate the intensity of the point process. Kernel estimators of density functions can be extended to obtain nonparametric estimates of the intensity of a point process (Cressie, 1991). This is similar to passing a moving window of size W , across the region and estimating the number of deer per unit area. The size of the window determines the amount of smoothing (i.e. bandwidth) and is of primary concern when estimating the intensity. Any reasonable kernel estimator will give close to optimal results. This process results in a three-dimensional surface depicting how the intensity, or the number of deer per unit area, changes over a bounded region, A .

We will then develop multiple regression models to relate landscape maps of primary environmental features including elevation, hydrology, vegetative cover, and land use variables to best fit values in porosity matrices (Table 2). We will also use secondary, derived indices of habitat fragmentation, perforation, and connectivity as independent variables in the analysis. We will use variable selection techniques described above to identify independent variables (spatial features) exerting the greatest control over changes in the distribution and abundance of diseased animals.

Model Experiments

Presuming that our analysis finds that patterns of land use affect spatial patterns of disease, we will use land-use change models developed for the region (Theobald and Hobbs, 1998) to project how shifts in land use will affect the future spatial distribution of the CWD. Our land use change model uses population projections from the State of Colorado Demographic Model to drive simulations of future residential development and associated infrastructure and its impacts on wildlife habitat (Theobald

Table 2. Independent variables used in regression analysis of the porosity grid.

<u>Environmental grids</u>
elevation
hydrology
vegetation
<u>Land use grids</u>
land use classes
population density
road density
housing density
average distance to road
<u>Derived grids</u>
index of habitat connectivity
index of habitat patch size
perforation index

et al., 1997; Theobald and Hobbs, 1998; Theobald et al., 1999). We will use this model to project future porosity grids and will use our best approximating model of disease dynamics to project how prevalence will change in the future. This approach will represent changes in prevalence over time in a spatially explicit fashion.

We will also modify hunting mortality to mimic reductions in hunting pressure that accompany increased urbanization. We will represent control strategies by adding simulated culling to achieved hunting mortality.

Expected Results

We are confident that we will be able to formulate a model of CWD epidemic dynamics that has strong support in the data. The appropriate level of detail in that model remains uncertain, but the data that will emerge from our field campaign will offer a solid basis for choosing that level of detail. Moreover, given that the human population is growing at a rapid pace in the epidemic area and given that residential development needed to accommodate that growth will affect the amount and configuration of habitat available to mule deer (Theobald et al., 1997), it is likely that we be able to identify effects of land-use change on disease dynamics. If these effects emerge, then we will be able project impacts of future development on the spatial configuration of CWD.

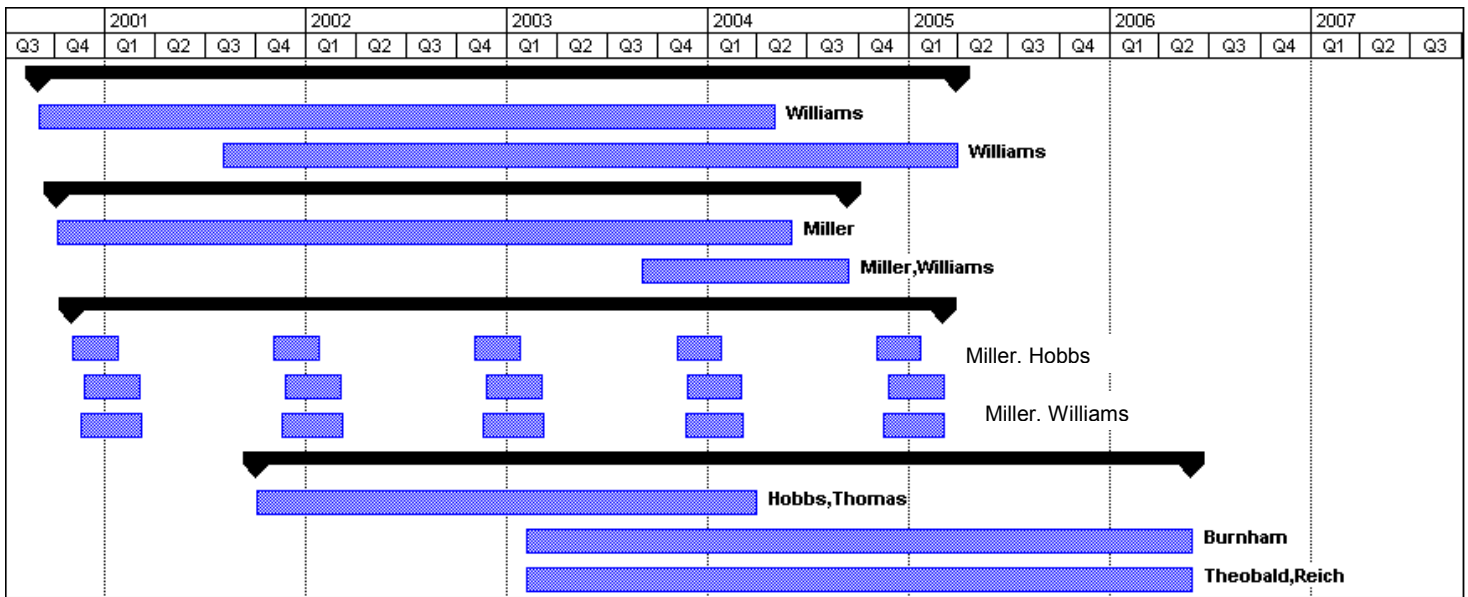
Potential Limitations/Pitfalls

Although the types of models we have proposed to use have enjoyed broad success in abstracting the essential dynamics of disease (Jeltsch et al., 1381; Barlow, 1993; Johansen, 1996; Lloyd Alun and May Robert, 1996), it may be that the structure we propose will be unable to adequately represent CWD. In this case, we have experience individual-based simulations of disease dynamics (Hobbs and Miller, 1992; Gross and Miller, submitted), and could modify our modeling efforts to employ these alternative, computer intensive approaches.

PROJECT ORGANIZATION AND MANAGEMENT

Scheduling of work and leadership roles are outlined in Fig. 8. Hobbs will direct the project and will participate in field and modeling studies. He has extensive experience in organizing and managing large, interdisciplinary research projects. Williams will lead the laboratory studies with the help of technicians. Miller will supervise technicians and a post-doc in conducting field studies. Reich and Theobald will lead acquisition spatial data and its analysis. Thomas will be responsible for providing numerical solutions to the differential equation models. Burnham and a graduate student will conduct model selection.

Figure 8. Scheduling and leadership roles for major tasks of project.



VERTEBRATE ANIMALS

Both mule deer and transgenic mice will be used in proposed laboratory studies. Relevant aspects of care and use for each species are described separately below.

Mule deer: All captive deer studies will be conducted at the Colorado Division of Wildlife’s Foothills Wildlife Research Facility (FWRP). The FWRP is located about 0.5 km west of Ft. Collins in Larimer County. This approximately 15 ha facility was established in the 1960s and expanded in the 1970s to support ongoing research on various aspects of native ruminant ecology, physiology, and management. The FWRP perimeter is double-fenced with 2.5 m game-proof woven wire and >2 m high-tensile electric or woven wire fences around paddocks. Spatial buffers of 6 m or more exist between the perimeter fence and all animal paddocks, and consequently there is no opportunity for fence-line contact with domestic livestock or free-ranging deer or elk. Isolation pens, alleyways, electronic scales, offices, surgical facilities, and postmortem facilities are available on site. Researchers, staff, veterinarians, technicians, and animal caretakers have extensive experience in the management and handling of captive wild ungulates, including mule deer. Facility access is limited to authorized personnel, and existing facility management policies preclude transfer of live ruminants from FWRP. Animal wastes from FWRP are composted and transferred periodically to a local landfill for burial; carcasses from all study animals will be submitted for complete necropsy followed by incineration. The CDOW FWRP is a USDA-registered animal research facility (USDA/APHIS Registration Number 84-R-0045), and all animal care and research programs are reviewed and approved by the CDOW Animal Care and Use Committee in accordance with federal animal welfare regulations.

Mule deer will come from one of two sources. Fifteen orphaned mule deer fawns will be acquired and bottle-raised using established protocols (Wild and Miller, 1991). In addition, a total of 60 3- to 6-month-old mule deer fawns will be captured from the Rocky Mountain Arsenal National Wildlife Refuge (RMANWR) and transported to the FWRF in Fort Collins, Colorado for use as experimental subjects. Previous surveillance (Miller et al., 1999, in review, and unpublished data) has revealed that RMANWR deer populations are not infected with CWD, thereby ensuring fawns will not be exposed to CWD prior to entering the experiment. For capture, fawns will be anesthetized with tiletamine HCl and zolazepam (Telazol[®]; 5 mg/kg) and xylazine HCl (2.5 mg/kg) delivered intramuscularly (IM) via projectile syringe (Miller, 1998). Additional xylazine (20-100 mg IV or IM) will be administered as needed to keep fawns sedated until they arrive at FWRF. Upon arrival at FWRF, residual sedation will be antagonized with IV yohimbine HCl (0.25 mg/kg).

Proposed live-animal uses:

In one study, bottle-raised mule deer will be orally-inoculated with CWD-infected brain tissue and used as sources of excreta for mechanistic studies of CWD transmission. These animals will be sampled every 6 months, and blood, saliva, urine and feces collected. For sampling, deer will be anesthetized with tiletamine HCl and zolazepam (Telazol[®]; 5 mg/kg) and xylazine HCl (2.5 mg/kg) delivered via hand or projectile syringe. Deer health will be monitored daily, and individuals showing clinical signs of CWD (or other serious health problems) will be euthanized as described below.

In a second study, wild-caught mule deer will be used to demonstrate the occurrence of indirect CWD transmission and to compare the intensities of direct (= animal-to-animal) and indirect transmission under controlled conditions. This controlled experiment will explicitly test the null hypothesis that occurrence, mean time to clinical onset, and mean infection intensity will not differ among groups of mule deer exposed to different potential sources of CWD infection. All deer will be observed daily by animal caretakers. Each subject deer also will be evaluated at least monthly by an attending veterinarian (Miller) experienced in recognizing clinical signs of CWD (Williams and Young, 1980, 1992), and subjectively scored (0 = not shown, 1=subtle, 2 = obvious) for behavioral changes, loss of body condition, ataxia, and salivation or polydipsia. Subject deer with clinical scores ≥ 3 will be regarded as clinical CWD cases and euthanized. Because animal-to-animal transmission within groups could easily confound transmission from environmental sources, all subject deer in an individual paddock will be sacrificed and subjected to complete necropsy and tissue sampling when the first clinical case occurs among subject deer in that paddock. Provided no clinical CWD cases occur in control paddocks, all subject deer in control paddocks will be sacrificed ≥ 12 mo after the last group of exposed subject deer is sacrificed.

Subject deer for both studies will remain in confined to study-specific paddocks (1-2 ha in size) throughout the respective studies. In addition to natural forage, alfalfa hay, pelleted supplemental diets (high-energy and “browser” rations), mineralized salt blocks, and water will be provided *ad libitum* in all paddocks as per standard feeding and husbandry protocols. Captive mule deer have been maintained at the FWRF for over 30 years, and FWRF staff and caretakers have extensive experience with captive mule deer husbandry and management.

Justification:

The natural host range of CWD is limited to mule deer, white-tailed deer, and elk. Of these species, mule deer appear to be most commonly affected, and therefore are the most logical species

for studies of natural CWD transmission mechanisms. Use of live animals is necessary in these experiments because suitable *in vitro* models and methods for CWD transmission are not available; moreover, data from these studies will be applied to improving parameter estimates in epidemic models, and consequently should have some basis in natural processes represented by live animals. Our sample sizes (n = 10 inoculated deer and n = 5 deer/exposure treatment group with 3 replicates/treatment) are the minimum necessary to ensure reasonable power in distinguishing among experimental treatments.

Veterinary care:

Two full-time veterinarians oversee all aspects of FWRF management and research animal care. Individual animals are observed daily by caretakers and/or attending veterinarians, and appropriate medical attention is provided to injured or ill individuals in accordance with established facility and experimental protocols by or under the supervision of the attending veterinarian.

Procedures for minimizing distress, pain, and discomfort:

In general, we will use anesthetic drugs in handling and sampling deer to minimize distress, pain, and discomfort. Details of anesthesia procedures are described above. Our handling and experimental procedures in and of themselves are not painful to the animals. However, extrapolating from the human spongiform encephalopathies, some pain may be present during the clinical course of the disease. Humans experience muscle atrophy, ataxia, dysphagia with emaciation, incontinence, ill-defined pain sensations, and behavioral changes such as depression and dementia (Collinge and Palmer, 1997). All of these behavioral as well as body changes are a result of the neurologic destruction that occurs during the clinical course of the disease. We are unable to control these changes because: 1) we do not want to mask the clinical signs of this disease so that we may properly record clinical course in conjunction with pathogenesis; 2) if we were to treat specific symptoms, then we may inadvertently change the outcome of the disease process; and 3) at this time, there is no known course of action that will stop the neurologic destruction caused by this disease. As the clinical disease progresses, obvious signs will develop, including some combination of excessive salivation, emaciation, difficulty walking, head and ear drooping, polydipsia, polyuria, and general unthriftiness (Williams and Young, 1992). Once these signs reach end-points prescribed in respective studies, the affected animal will be euthanized as described below.

Euthanasia methods:

Criteria for euthanasia are described above. At the time of sacrifice, deer will be anesthetized with Telazol[®] (5 mg/kg) and xylazine HCl (2.5 mg/kg) delivered via hand or projectile syringe. We will collect necessary samples, then administer about 400-1000 mEq KCl intravenously to induce cardiac arrest. In some cases, deer may be euthanized via gunshot (>0.223 caliber projectile) to the neck if anesthetic delivery is infeasible. Both approaches are consistent with recommendations of the AVMA Panel of Euthanasia; the use of KCl in this situation is acceptable because animals will be rendered unconscious with Telazol[®] and xylazine prior to KCl administration, thereby rendering the cerebral cortex nonfunctional and precluding pain perception. These approaches are preferred because others (e.g., barbiturate overdose) may compromise the use of deer tissues in subsequent bioassay and related studies.

Transgenic mice: All transgenic mouse studies will be conducted at the University of Wyoming's Department of Veterinary Sciences facilities at the Wyoming State Veterinary Laboratory. Animal rooms are available on-site, and laboratory animal care is conducted in accordance with established NIH guidelines. The WSVL necropsy laboratory, with adjacent specimen photography and preparation areas, is fully equipped. Additional support facilities include a histology laboratory, molecular biology laboratory, and electron microscopy suite.

Proposed live-animal uses:

The purpose of this study is to evaluate the contribution of various biological materials to the transmission of CWD. We will use transgenic mice that express mule deer PrP for bioassays of feces, urine, saliva, and placenta to determine if they contain the infectious agent (PrP^{res}). At each 6-month time step in the incubation of CWD, we will use six groups of mice. The four principal groups will test tissues/fluids for the presence of PrP^{res}; in addition, there will be a positive and negative control group. There will be eight time steps (6 months of CWD incubation, to 48 months of CWD incubation). We are using 6-month time steps so that we can understand the temporal dynamics of possible shedding of the CWD prion. For inoculations, weanling mice will be anesthetized with 50 mg/kg ketamine plus 5 mg/kg xylazine given intramuscularly (Carpenter, Mashima, and Rupiper, 1996. Exotic Animal Formulary, Greystone Publications, Manhattan, Kansas). The skin over the cerebral cortex will be shaved and scrubbed for surgery. The mice will be inoculated intracerebrally with 0.02 ml of tissue/fluid preparation in to the cerebral cortex using a 26 g needle on a tuberculin syringe. To improve the efficiency of transmission, 0.100 ml of the preparation will be given intraperitoneally. Mice will be allowed to recover from anesthesia and will be housed routinely. They will be monitored daily for evidence of clinical disease.

Justification:

Bioassays are considered the most sensitive method for detection of PrP^{res}. Typically these bioassays are conducted in the host species, which in this case would be mule deer. However, in order to detect PrP^{res} in all the samples at all the time periods necessary we would have to use a very large number of mule deer. To avoid that, we are collaborating with researchers elsewhere that are developing transgenic mice that have the mule deer PrP gene and express mule deer PrP. Thus they can serve as sensitive indicators of the presence of PrP^{res} in the test material. [If we are unable to obtain the transgenic mice needed for these studies, we may then have to use deer (we will submit a revised protocol for ACUC review and approval in that event).] We have considered other methods for detection of PrP^{res} in various biological materials and western blots will also be conducted. However, previous studies, by many researchers working with the prion diseases have shown that at the current time, bioassay is the most sensitive method.

This research has not be conducted elsewhere and there is no information in the literature that addresses the mode of transmission of CWD, based on review of the literature on CWD and other prion diseases. We chose groups of 10 mice each to have adequate numbers of animals to assess the presence of PrP^{res}, which we expect will be present in low amounts in the materials we will be testing. Also, the mice will be maintained for 3 years, thus some mice may succumb due to intercurrent disease or old age and in order for the assays to be valid we must have appropriate numbers of mice alive for the duration of the test.

Veterinary care:

Dr. Williams will provide or oversee veterinary care for study mice under established institutional protocols. Dr. Williams has about 23 years of experience in the laboratory study of infectious diseases of animals, and is experienced in conducting studies using similar techniques to those proposed for this work.

Procedures for minimizing distress, pain, and discomfort:

Painful procedures will be conducted under anesthesia (50 mg/kg ketamine plus 5 mg/kg xylazine IM; Carpenter, Mashima, and Rupiper, 1996. Exotic Animal Formulary, Greystone Publications, Manhattan, Kansas). Mice showing neurological signs, or signs of other intercurrent disease, will be euthanized as described below.

Euthanasia methods:

If mice develop clinical signs of CWD, they will be euthanized by overdose of metafane inhalation in a glass chamber. At the end of the study (3 years post inoculation), all mice will be euthanized as described above. This approach is consistent with recommendations of the AVMA Panel of Euthanasia.

Wild, M. A. and M. W. Miller.1991. Bottle-raising wild ruminants in captivity. *Colorado Division of Wildlife Outdoor Facts*, 1-6.