CWD Annual Report 2010-2011

Activities

Background
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 caused by an accumulation of a proteinase-resistant prion protein (*PrP*) in the brain of affected individuals. Similar diseases include scrapie in sheep and goats, bovine spongiform encephalopathy in cattle and Creutzfeldt-Jacob disease in humans, all of which cause neural degeneration and, inevitably, death.

When we received our first Ecology of Infectious Disease award in August of 2000, the known distribution of CWD was limited to a single cluster of populations of mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*) and elk (*Cervus elaphus*) arrayed in northeastern Colorado and Southeastern Wyoming. Today, CWD has been found in free-ranging cervid populations in 13 states and provinces in North America and as well as in over 80 captive herds (Figure 1). The disease has also been found in South Korea has also spread to moose (*Alces alces*).

CWD is transmitted horizontally, likely via oral exposure to saliva, blood or to residual excreta or carcass remains. Oral infection via urine or feces cannot be ruled out. Evidence suggests that infectious materials can persist in the environment for years and soil particles appear to represent a plausible environmental reservoir for prion infectivity. Recent, experimental findings show remarkable amplifying effects of clay soils on infectivity. Binding infectious prions to montmorillonite (clay) soils increased their infectious titer by a factor of 680 relative to an unbound agent.

We characterized the prion protein (*PrP*) gene in 1,482 free-ranging mule deer from Wyoming and Colorado, finding dimorphisms at codons 20 (aspartate/glycine) and 225 [serine (S)/phenylalanine (F)]. Polymorphism at codon 225 correlated with CWD status: the odds that deer of the *SS* genotype were CWD-infected were 30 times greater (95% confidence interval = 4–213) than for *SF* deer. These results suggest that the *SF* genotype conveys resistance to CWD, a result that resembles findings on genetic controls on susceptibility in other cervids. The *F* allele varies in frequency from 0 to as high as 11% in populations sampled in Colorado and Wyoming, with higher frequencies in Colorado than in Wyoming. In Colorado, as many as 20% of deer have the *SF* genotype, based on hunter samples from northeastern Colorado.

Simple, compartment models based on systems of differential equations explained trajectories in observations of CWD-induced mortality in captive populations (Figure 2). However, our ability to portray dynamics of free-ranging populations infected with CWD remains rudimentary.

Overview of Research

Aims

A great deal is known about the effects of CWD on individual deer. There is also substantial understanding of population level patterns of the disease over time and space. However, population level processes are poorly understood. To illuminate those processes, we are conducting a field study using multi-state, mark-capture-recapture methods to estimate survival probabilities (φ) and probabilities of infection (**g**) in populations of mule deer infected with CWD.

Aim 1: Provide a case example of a novel, general approach for assimilating data with models of emerging infectious diseases that meets the three needs outlined above.
Aim 2: Evaluate support in data for competing models of transmission of CWD and estimate the basic reproductive rate of the disease.
Aim 3: Reveal demographic, genetic, and environmental sources of heterogeneity in disease transmission.
Aim 4: Use the models developed under Aim 1 and 2 to evaluate the consequences of disease for the trajectories of populations infected with proteinase resistant prions.

Sensitivity Analysis of High-Dimension Models

Consider the following system of parameterized nonlinear first-order ordinary differential equations,

(see Equation 1)

where $\boldsymbol{x} \in \mathbb{R}^M$, $\boldsymbol{p} \in \mathbb{R}^K$ and the initial conditions $\boldsymbol{z} \in \mathbb{R}^M$

We define the sensitivity of the i^{th} variable with respect to the k^{th} parameter, $S_{i,k}$ as

(see Equation 2).

Differentiating with respect to the k^{th} parameter p_k (and reversing the order of differentiation w.r.t. t and w.r.t. p_k on the LHS) gives

(see Equation 3).

The most difficult part is to evaluate the terms in red in the above equation. SENSAI uses Maple and the (free) Matlab-Maple toolbox to write the necessary Matlab routines.

Example Calculation using SENSAI

Consider a classical SIR model of plague in rats with two extra equations modeling the number of fleas per rat and the number of infectious fleas in the environment (Buzby et al, 2008). SENSAI can provide the solution to the ODE model as well as sensitivities and elasticities of every state with respect to every parameter, including initial conditions. Also, a quantity of interest (QOI), for example, the proportion of infected rats, can be defined and the sensitivities and elasticities of this can also be computed. Here is a small sample of some of the information SENSAI produces (Figures 3 & 4).

SENSAI can input several equations with several parameters and can thus compute sensitivities, etc., for most models. Finally, SENSAI gives us the capability to build and analyze multistage disease models with single locus multi-allele genetics.

Mark-recapture Simulations

We propose to model the mark-recapture data using a multinomial state-space model with a hidden process level and an observed data level. The hidden disease state (x) and observed state (y) of each animal for each year are represented as vectors, in which each cell represents each of 7 states: 1. susceptible, 2. infected, 3. died susceptible, 4. died infected, 5. out of study, 6. dead but infection status unknown, 7. unobserved. Our state-space model includes a matrix to transition the hidden data from year t-1 to year t, and a matrix to transition the hidden data from year t to the observed data in year t (see Equations 4 and 5).

Using this model, conducted a lengthy series of simulations in an effort to make the best use of project resources. Each simulation consisted of a different combination of parameter levels, including disease transmission, mortality, and recapture probabilities. Differing new animal capture scenarios were simulated, as well, particularly total sample size and new collar deployment strategies (e.g. all new captures deployed in the first year versus deploying new collars over several years).

As a measure of prediction accuracy, the widths of highest posterior density intervals for mortality and disease transmission probabilities were compared across scenarios. The simulations provide us with a minimum prediction accuracy baseline for a set of study parameters (see Figure 5)—accuracy that we hope to improve by incorporating informative priors and covariate data.

One surprising outcome of the simulation study was that studying deer post-mortem proved to be impractical. If possible, deceased animals were tested for chronic wasting disease, a strategy made difficult by rapid scavenging. The simulation study suggested that the information gained by this tactic was minimal and insufficient to justify the effort and cost required (see Figure 6).

Genetic Measurements

We are examining the role of genetics in creating heterogeneity in transmission by incorporating genotypic information as covariates in the analysis. First, we have determined tentative *PrP* genotypes of deer captured in the first two years, using a simple restriction fragment analysis (see Figure 7). The *PrP* gene is variable in at least seven cervid species, and variation in the *PrP* gene in mule deer relative to other cervids is well established. Genotypic information (225*SS, SF, or FF*) will be used in population genetic analyses and as covariates in the analysis of sources of heterogeneity in transmission. Genetic analyses will have high statistical power because hundreds of deer will be sampled. DNA has been extracted from blood using standard protocols; PCR protocols and scoring of alleles have been standardized.

Next, all deer will be genotyped for a series of microsatellite markers and the control region of the mitochondrial DNA (mtDNA), to: 1) calculate relatedness among deer; and 2) assess movement of males relative to females. Microsatellite markers will be used to estimate relatedness of individuals within maternal groups. Tetranucleotide (CATC, TAGA) microsatellites, developed by the California Department of Fish and Game (GenBank AF102240–AF102260) are sufficiently diverse to allow accurate estimation of

relatedness. Each deer will be genotyped for at least ten markers, which will provide adequate power for both estimating relatedness between deer and determining population assignment. The ten markers should yield >99% probability of identity, calculated as the probability of (not) sampling the same multilocus genotypes within maternal groups, given the total probability of sampling any possible genotype twice. Because of maternal inheritance, mtDNA analysis will allow us to further distinguish between gene flow by male dispersal (deduced from microsatellite markers), and genetic structure and stability of local female groups (in the cases where microsatellites show no differentiation, but mtDNA does). We will sample the mtDNA control region, which is highly variable in mammal populations.

Findings

Mark-recapture Studies

We captured 140 female deer during January 2010 including 125 adults ($\geq$ 1.5 years old) and 15 juveniles (6 months of age). Surviving deer were targeted for recapture during January 2011 and we successfully handled 89 individuals. We additionally captured 76 adult females, 5 juvenile females, and 10 adult males. Disease status was determined for adults during handling. We specified the posterior distribution of adult female prevalence during 2010 as $P(p_{2010}|y_{2010},n_{2010})$=beta(5,122) which provided a parameter model for the posterior distribution of adult female prevalence during 2011 specified as $P(p_{2011}|y_{2011},n_{2011})$=beta(14,278). A separate analysis of adult male prevalence was completed where we specified the posterior distribution as $P(p_m|y_m,n_m)$=beta(3,9). Posterior median and 95% credible intervals of prevalence during 2011 were 0.05 (0.03. 0.08) for females (Figure 8) and 0.24 (0.06, 0.52) for males.

Sampling of the rectoanal mucosa-associated lymphoid tissue (RMALT) provides an antemortem test of CWD status. The number of lymphoid follicles obtained in a sample varies and it is becoming accepted that a minimum of $\geq$6 follicles is necessary for a diagnostic test. We found that the number of follicles detected during subsequent handling of individuals decreased (Figure 9) and we adjusted our capture-recapture model used in our data simulations.

We defined π_l as the live recapture probability, p as the probability a lymphoid follicle is positive in an infected deer, π_m as the mortality recapture probability, ϕ_s as the survival probability of susceptible females, ϕ_i as the survival probability of infected females, and ψ as the probability of infection. Juvenile and adult survival of susceptible deer was differentiated using the equation $\text{logit}(\phi_s) = \beta_0 + \beta_1 x$ where x was a 0,1 indicator variable for juveniles. The cell for false negatives in the matrix relating true states and observations became the product of π_l and $(1-p)^{ni}$ where n_i represented the number of follicles obtained for an individual. By the same logic the cell for true positive deer became $\pi_l - \pi_l (1-p)^{ni}$. We also specified a parameter model for p as

(see Equation 6)

where y_i and n_i were numbers of positive and total follicles in observed infected deer.

We are the first to use mark-recapture models to estimate the probability of infection of deer infected with CWD. Our initial estimate of the posterior median and 95% credible interval of infection probability is 0.06 (0.02,0.08; Figure 10). Recovering this parameter is an important first step in understanding the underlying transmission process.

Adult female survival of infected deer was 0.48 (0.14, 0.83) and we found a 0.94 probability that survival of infecteds was less than susceptibles. We estimated the survival of susceptible adults as 0.78 (0.70, 0.85) and survival of juveniles as 0.64 (0.24, 0.93). Probability that a follicle was positive in an infected deer was 0.88 (0.83, 0.92) indicating high test sensitivity after infection has progressed to the terminal nodes of the lymphatic system. Recapture probability of live individuals was 0.86 (0.82, 0.89) and 0.54 (0.38, 0.70) of dead individuals.

1) New participants:
Graduate student in the CSU Statistics Department: Lenae Andersen working on the Rocky Mountain High School student project

Jessica Nell, CSU undergraduate: data analysis and poster (see below)

2) Training and development:
We have recruited four graduate students, two in ecology and biology, one in statistics, and one in mathematics. Numerous undergraduate students are also involved.
These students are receiving interdisciplinary training throughout working closely together to implement project goals. Biology and ecology students are participating in field work and math and statistics students are doing mathematical and statistical modeling.

Undergraduate Research Experience
First-year Honors Undergraduate Research Scholars Emily Dommermuth, Katharine Fielding, Nicholas Bartush, and Stephanie Richert worked with Co-PI Boone throughout the academic year. In the fall semester the students learned about the scientific method, what it means to be a scientist, and the nature of data. In the spring semester, students learned introductory concepts to geographic information science. The students have learned about the projects they wished to be most closely associated with (this project tracking mule deer, EF-0914489, PI: Hobbs, and DEB-0919383 tracking migratory wildebeest, PI: Boone), and each has gained experience in using the equipment helping our team track mule deer. The students on the Laramie Foothills Mule Deer Project, Nicholas Bartush and Emily Dommermuth, participated in capture activities, and in addition to gaining extraordinary experiences, helped the larger team greatly.

In the spring semester, the students began a larger research effort, testing the accuracy of a Global Positioning System (GPS) collar used on mule deer.
Over several months the students moved a collar to 15 locations, recording the true position of the location using a hand-held GPS unit. The students worked with Boone to analyze the results, then prepared a poster that was displayed at the 'Celebrate Undergraduate Research and Creativity' Symposium on the CSU campus in April 2010.

The students received positive comments on their poster. The testing of the collar is somewhat informative to the larger project. But most importantly, the students learned about project design, GIS, simple statistical analyses, and the presentation of information to audiences. The students will continue to work with Dr. Boone throughout their undergraduate education.

We used a part of the CWD data set in Hoeting's STAT472 course "Statistical consulting". As part of this course, 3 students (1 undergraduate and 2 graduate students) analyzed the data to determine whether different medication regimes reduced stress levels in the deer in the study during the deer captures in January 2011. The undergraduate produced a paper and a poster that was presented at a campus-wide research symposium.

3) Outreach:
Hobbs led a training session on CWD for interpretive staff at Rocky Mountain National Park as well as for Master Naturalists at the City of Fort Collins.
We have contributed educational materials and interpretive signage regarding CWD and the study to the City of Fort Collins and Larimer County. We have initiated a collaboration with four high school teachers who will help us with field investigations and with outreach to the local community. They are participating in all project meetings and will become familiar with all aspects of the research with the ultimate goal of developing curriculum for biology and statistics classes using the research.

We have created a web reporting form to provide people living in our study area a quick and efficient way of sharing information on any of our collared deer they happen to see. The form contains fields for location information, identification information, and information on the physical appearance and behavior of the deer. All information collected is placed in a database that can be accessed by research team members for further analysis. Currently, work is being done to create an interactive map to accompany the sighting form, which the observer can use to merely click on the spot where they saw the deer and the map will then deposit the coordinates into the database. This allows the observer to provide an accurate location, without having to type in specific UTM or latitude/longitude coordinates. The sighting form is currently available on our project's website.

We have held two community public information meetings in May and December, 2010, as well as three meetings with our landowner advisory committee.

8-18-2010 Hobbs gives talk on project to City of Fort Collins Natural Resource Department staff
8-26-2010 Hobbs gives talk on project to Larimer County Natural Resources Advisory Board
1-12-2011 Hobbs meets with Starry Night subdivision landowners to discuss their concerns about helicopter capture

North Forty News articles on project:

December 2009

North Forty News newspaper article on project: CSU launches new study on chronic wasting disease
http://www.northfortynews.com/Archive/A20091202_CWDstudyCSU.htm

March 2010

North Forty News newspaper article on project: Researchers capture more deer for CWD study
http://www.northfortynews.com/Archive/A201003photo_03_CWDstudyDeerCapture.htm

January 2011

North Forty News newspaper article on project: CWD study seeks deer spotters (archive link broken)

February 2011

North Forty News newspaper article on project: Owl Canyon residents protest deer capture techniques (archive link broken)

We have four high school science teachers and one community college science teacher involved in the project. We are working on creating a day-long interactive workshop for high school students in the fall to expose them to all facets of the project including modules in genetics, veterinary medicine, statistics and telemetry.

4) Publications or products:

Journal Articles

Dulberger, J., N. T. Hobbs, H. M. Swanson, C. J. Bishop, and M. W. Miller. 2010. ESTIMATING CHRONIC WASTING DISEASE EFFECTS ON MULE DEER RECRUITMENT AND POPULATION GROWTH. Journal of Wildlife Diseases 46:1086-1095.

Hobbs, N. T. and K. Ogle
Introducing model data assimilation to students of ecology
In press: Ecological Applications

LaDeau, S. L., G. E. Glass, N. T. Hobbs, A. Latimer, R. S. Ostfeld Data-model fusion to better understand emerging pathogens and improve infectious disease forecasting
In press: Ecological Applications

Tavener, S., M. Mikucki, S. G. Field, M. F. Antolin. Transient sensitivity analysis for nonlinear population models. Methods in Ecology and Evolution.
Article first published online: 25 MAR 2011

Wild, M. A., N. T. Hobbs, M. S. Graham, and M. W. Miller. 2011. THE ROLE OF PREDATION IN DISEASE CONTROL: A COMPARISON OF SELECTIVE AND NONSELECTIVE REMOVAL ON PRION DISEASE DYNAMICS IN DEER. Journal of Wildlife Diseases 47:78-93.

Other Publications

Nell, Jessica (2011) "Measuring Stress Levels in Deer", Celebrate Undergraduate Research and Creativity, Colorado State University, Poster.

5) Contributions:

* Contributions within Discipline
We are developing modeling approaches that we believe will become standard in many studies of infectious disease, particularly diseases of wildlife.

* Contributions to Other Disciplines

* Contributions to Human Resources Development
We have engaged graduate and undergraduate students and provided unusual opportunities to participate in an interdisciplinary project.

* Contributions to Resources for Research and Education
We are working on a field day where we will invite high school students to come learn about many aspects of the project, including veterinary medicine, genetics, statistics, telemetry, and animal and disease ecology. The modules developed for this field day will feed into lesson plans for high school students on subjects related to disease ecology.

* Contributions to other aspects of public welfare beyond science and engineering, such as commercial technology, the economy, cost-efficient environmental protection, or solutions to social problems

Activities: Figures and Equations

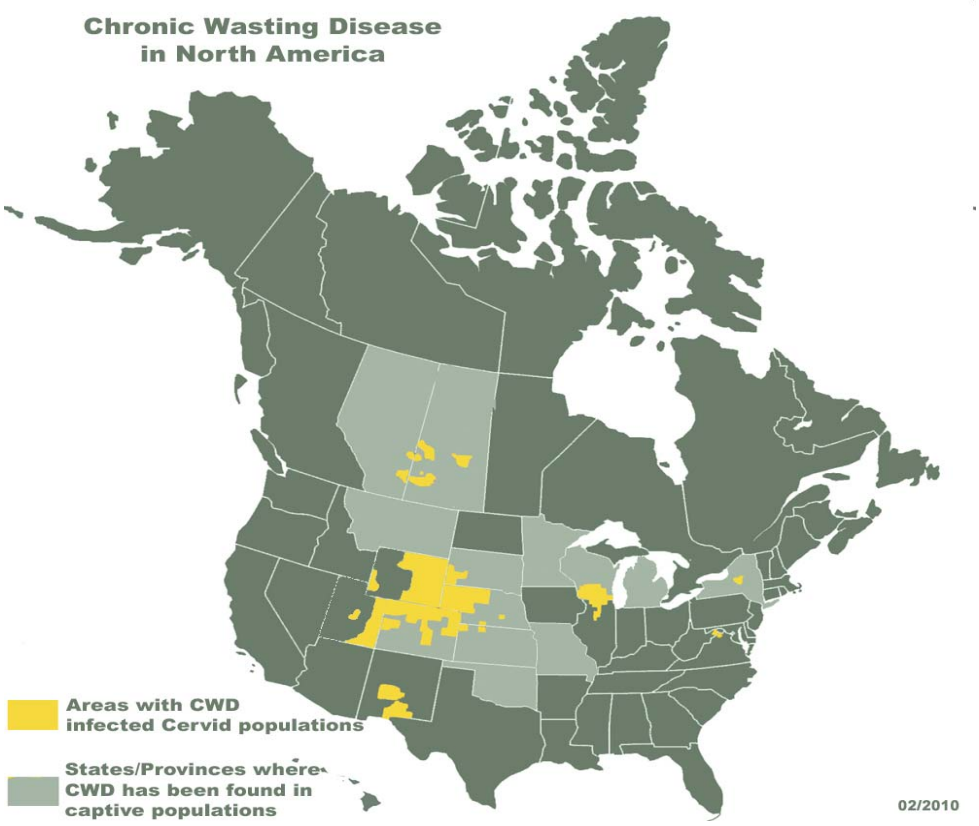

Figure 1. Distribution of CWD in North America. Chronic Wasting Disease Alliance (www.cwd-info.org)

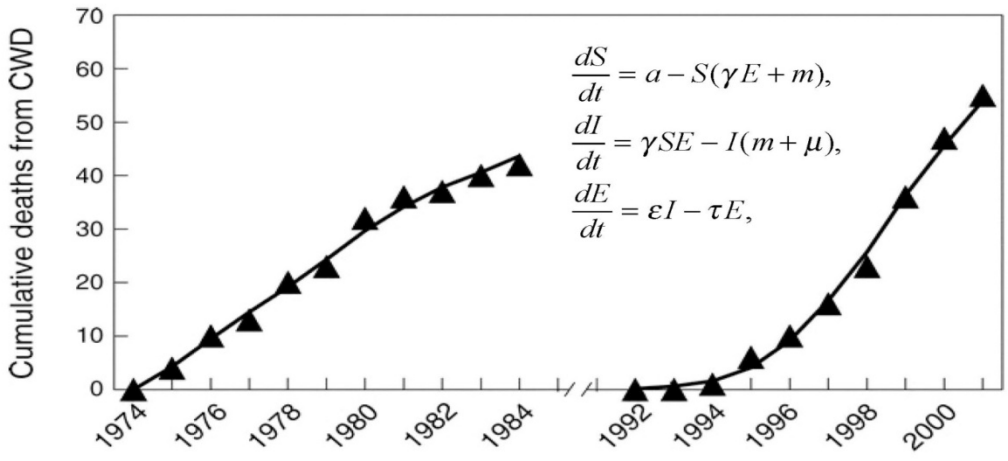

Figure 2. Fit of environmental transmission model to time series of cumulative mortality in two epidemics of chronic wasting disease.

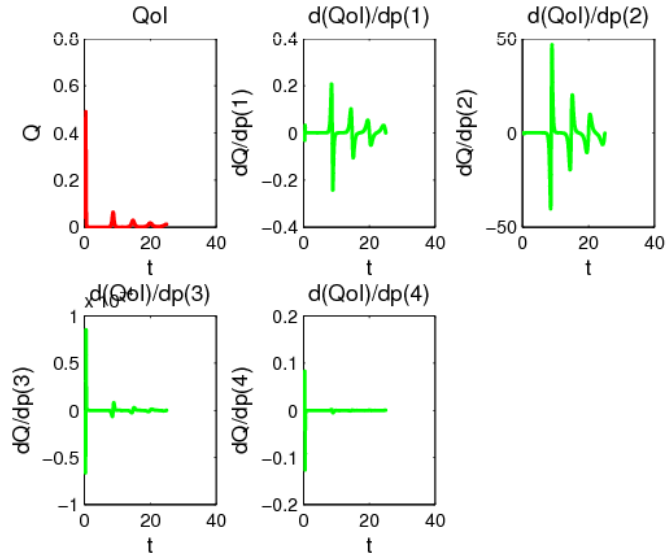

Figure 3: Solution and sensitivities of QOI with respect to all parameters at all times.

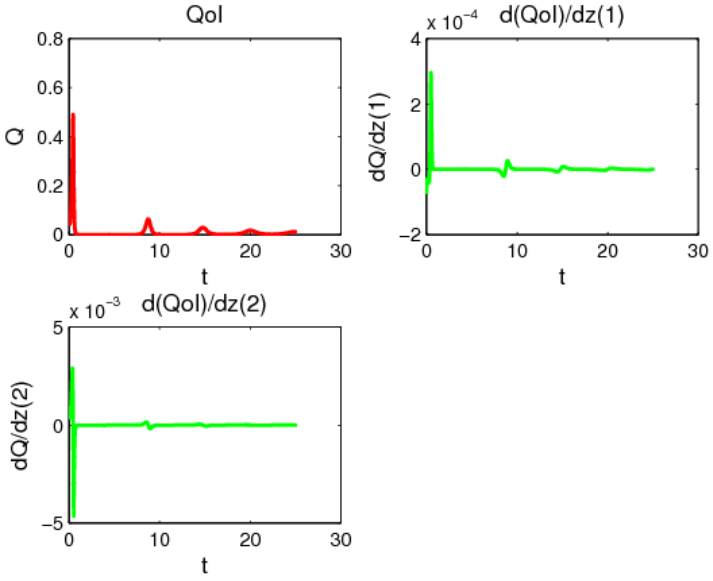

Figure 4: Solution and sensitivities of QOI with respect to initial conditions at all times.

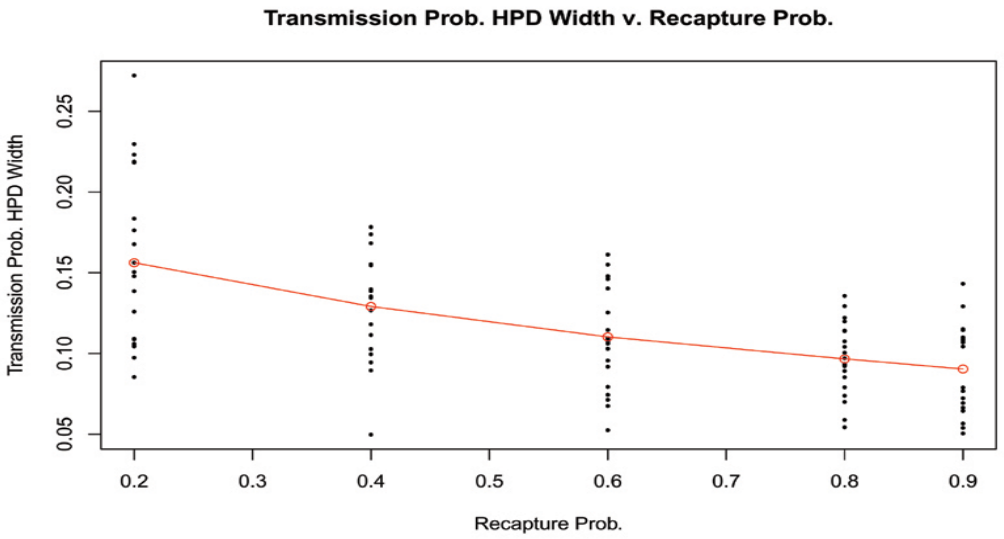

Figure 5: Simulation results for transmission probability estimation accuracy for varying recapture probabilities.

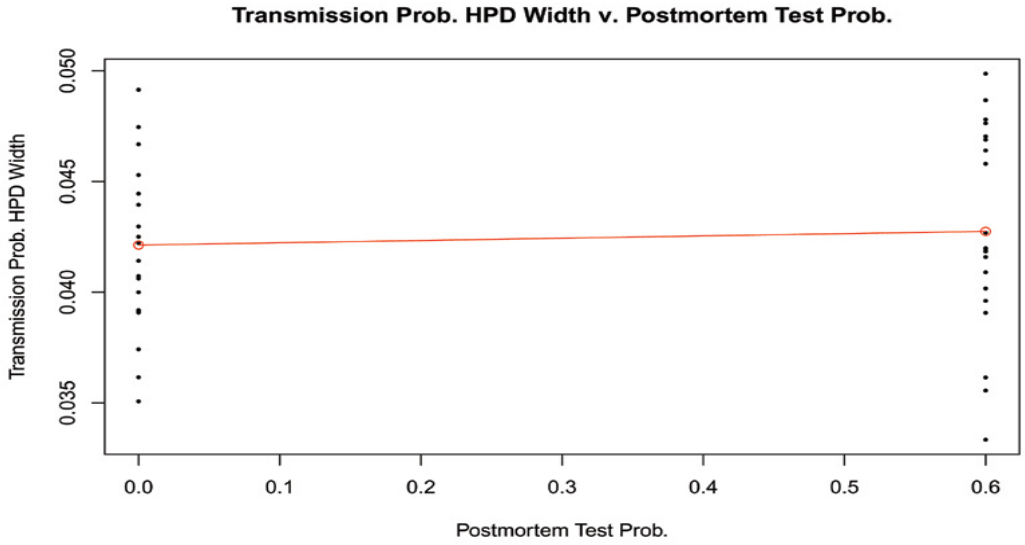

Figure 6: Simulation results for transmission probability estimation accuracy for postmortem test probability zero versus postmortem test probability 0.60.

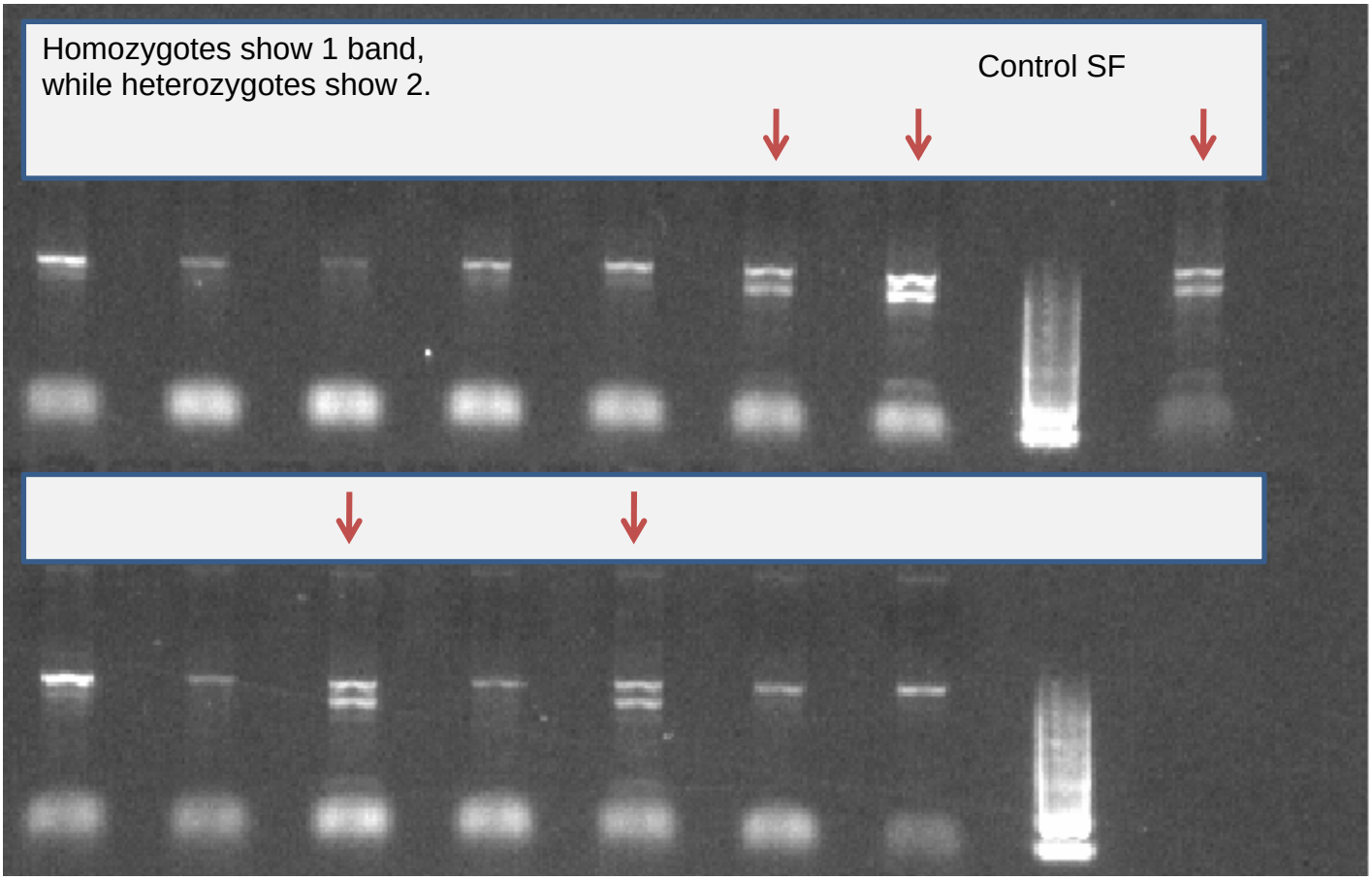

Figure 7: The complete open reading frame of *Prnp* plus 25 bp of 5' and 53 bp of 3' flanking sequences in the PrP-coding region were amplified using PCR in 30µl reactions. The PCR products were then digested with EcoRI for 3hours at 37°C.

Equation 1

$$\left.\begin{aligned} \dot{\mathbf{x}}(t, \mathbf{p}) &= \mathbf{f}(\mathbf{x}(t, \mathbf{p}), \ \mathbf{p}) \\ \mathbf{x}(0) &= \mathbf{z} \end{aligned}\right\}$$

Equation 2

$$S_{i,k} = \frac{\partial x_i}{\partial p_k}.$$

Equation 3

$$\left.\begin{aligned} \frac{d}{dt}\left(\frac{\partial x_i}{\partial p_k}\right) &= \sum_{m=1}^{M}\left(\frac{\partial f_i}{\partial x_m}\,\frac{\partial x_m}{\partial p_k}\right) + \frac{\partial f_i}{\partial p_k} \\ \frac{\partial x_i}{\partial p_k}(0) &= 0 \end{aligned}\right\}, \quad i = 1, \ldots M,\ k = 1, \ldots K.$$

Equation 4

$$x_{i,t} \sim Multinomial(1, Mx_{i,t-1})$$

where

$$M = \begin{bmatrix} \phi_{sus}(1-\phi_{trans}) & 0 & 0 & 0 & 0 \\ \phi_{inf}\phi_{trans} & \phi_{inf} & 0 & 0 & 0 \\ (1-\phi_{sus})(1-\phi_{trans}) & 0 & 0 & 0 & 0 \\ \phi_{trans}(1-\phi_{inf}) & 1-\phi_{inf} & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 1 \end{bmatrix}$$

for $i = 1, ..., n$ (n = total number of deer)
for $t = T_i^* + 1, ..., T$ (T_i^* = first year animal i is in study, T = total number of study years)

- ϕ_{trans} is the probability that a susceptible becomes infected
- ϕ_{sus} is the probability that a susceptible survives
- ϕ_{inf} is the probability that an infected survives

Equation 5

$$y_{i,t} \sim Multinomial(1, Dx_{i,t})$$

where

$$D = \begin{bmatrix} p_{sus} & 0 & 0 & 0 & 0 \\ 0 & p_{inf}(1-y_{t-1,i,2}) + y_{t-1,i,2} & 0 & 0 & 0 \\ 0 & 0 & p_{dead} & 0 & 0 \\ 0 & 0 & 0 & p_{dead}(1-y_{t-1,i,2}) + y_{t-1,i,2} & 0 \\ 0 & 0 & 0 & 0 & 1 \\ 0 & 0 & 1-p_{dead} & (1-p_{dead})(1-y_{t-1,i,2}) & 0 \\ 1-p_{sus} & (1-p_{inf})(1-y_{t-1,i,2}) & 0 & 0 & 0 \end{bmatrix}$$

for $i = 1, ..., n$ (n = total number of deer)
for $t = T_i^* + 1, ..., T$ (T_i^* = first year animal i is in study, T = total number of study years)

- p_{sus} is the probability of observing a susceptible animal
- p_{inf} is the probability of observing an infected animal
- p_{dead} is the probability of observing the CWD status of a deceased deer

Findings: Figures and Equations

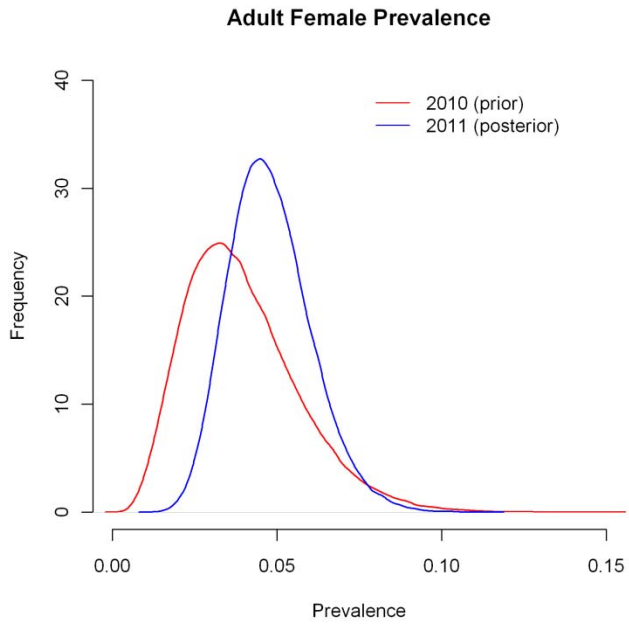

Figure 8: Prior (red) and posterior (blue) distributions of CWD prevalence in adult female deer during January 2011.

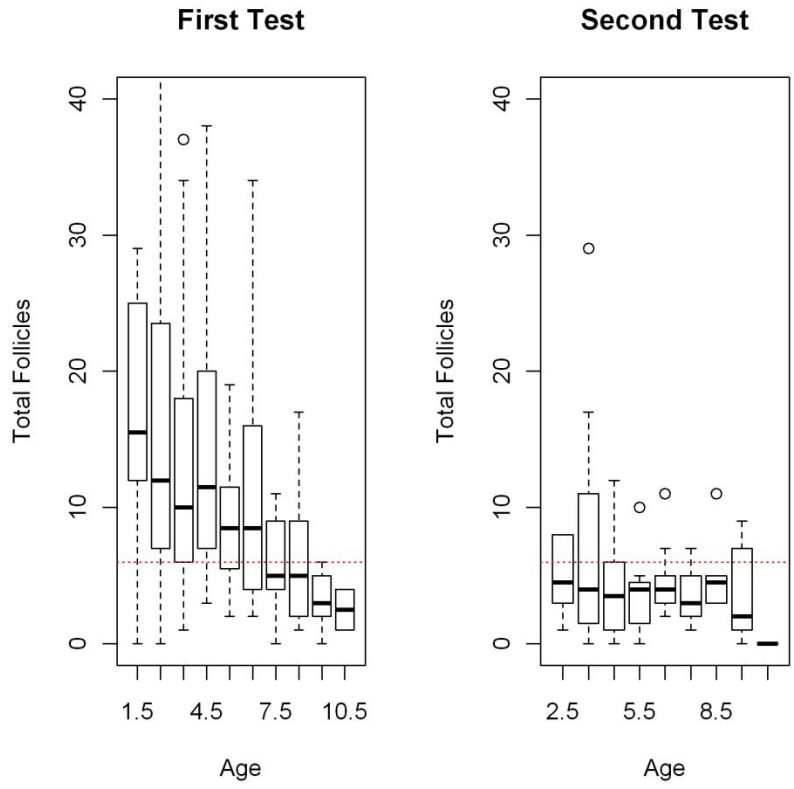

Figure 9: Summary of lymphoid follicles obtained during initial (2010) and repeat (2011) sampling of same individual deer.

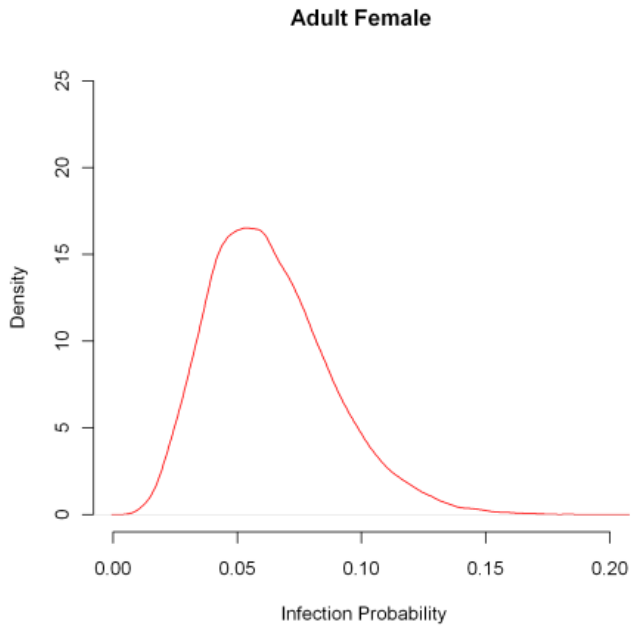

Figure 10: Posterior distribution of infection probability of adult female deer during January 2010 - 2011.

Equation 6

$$P(p \mid \mathbf{y}, \mathbf{n}) \propto \prod_{i=1}^{15} binomial(y_i \mid p, n_i) \times beta(p \mid 1,1)$$