AGE AND REPEATED BIOPSY INFLUENCE ANTEMORTEM PRP<sup>CWD</sup> TESTING IN MULE DEER (ODOCOILEUS HEMIONUS) IN COLORADO, USA

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ABSTRACT: Biopsy of rectal mucosa–associated lymphoid tissue provides a useful, but imperfect, live-animal test for chronic wasting disease (CWD) in mule deer (Odocoileus hemionus). It is difficult and expensive to complete these tests on free-ranging animals, and wildlife health managers will benefit from methods that can accommodate test results of varying quality. To this end, we developed a hierarchical Bayesian model to estimate the probability that an individual is infected based on test results. Our model was estimated with the use of data on 210 adult female mule deer repeatedly tested during 2010–14. The ability to identify infected individuals correctly declined with age and may have been influenced by repeated biopsy. Fewer isolated lymphoid follicles (where PrP<sup>CWD</sup> accumulates) were obtained in biopsies of older deer and the proportion of follicles showing PrP<sup>CWD</sup> was reduced. A deer's genotype in the prion gene (PRNP) also influenced detection. At least five follicles were needed in a biopsy to assure a 95% accurate test in PRNP genotype 225SS deer.

Key words: Bayesian, capture–mark–recapture, chronic wasting disease, mule deer, prion, test sensitivity.

INTRODUCTION

Developing effective strategies for managing wildlife diseases requires identifying relationships between hosts and pathogens (Dobson and Foufopoulos 2001; McCallum et al. 2001). Accurate diagnosis of infection status is a necessary first step for inferences about disease (McClintock et al. 2010; LaDeau et al. 2011). The veterinary and medical fields have developed statistical techniques for estimating true infection status when diagnostic tests are imperfect. Such techniques rarely are applicable to wildlife diseases because little is generally known about the underlying prevalence of disease in the population, and reference tests as well as repeated independent tests of disease status are not available (Greiner and Gardner 2000; Toft et al. 2005; Lachish et al. 2012). Furthermore, capture and testing of individuals is inherently difficult and costly, and the information available is often limited to one test or observation of a sick animal (Morner et al. 2002).

Chronic wasting disease (CWD) is a naturally occurring prion disease found in free-ranging elk (Cervus elaphus), mule deer (Odocoileus hemionus), white-tailed deer (Odocoileus virginianus), and moose (Alces alces) in North America (Williams and Young 1992; Baeten et al. 2007). Prion diseases are believed to be caused by the accumulation of a misfolded variant of native prion protein (PrP), PrP<sup>CWD</sup>, which leads to neurodegeneration and ultimately death (Williams and Miller 2002; Sigurdson 2008). PrP<sup>CWD</sup> accumulates in tissues of the lymphatic system early in the infection process, particularly in lymphoid follicles (Fox et al. 2006). Consequently, PrP<sup>CWD</sup> can be detected in live animals through
biopsy of tonsil or rectal tissue, which is tested by immunohistochemistry staining (Wild et al. 2002; Wolfe et al. 2002, 2007). Distribution of PrP CWD is not uniform among lymphoid follicles either in tonsil or gut-associated lymphoid tissues, particularly early in the disease course (Fox et al. 2006). Although one positive follicle is regarded as sufficient to classify an individual as infected, the interpretation of negative results can be more difficult. PrP CWD could occur within a tested animal, but remain undetected if only a small number of follicles are observed (Wolfe et al. 2002). Consequently, examining six or more follicles within a biopsy has been regarded as necessary to assure detection of infected individuals early in the disease course (Wolfe et al. 2002; Keane et al. 2009; Spraker et al. 2009a, b).

Although logical, using a set lymphoid follicle threshold for interpretability discards test results that actually could provide meaningful information about the infection status of an individual. The probability that a test produces a false negative depends (at minimum) on the true infection status of the animal, the number of follicles tested, and the proportion of follicles that are positive (Fox et al. 2006; Wolfe et al. 2007, 2014). Tissue biopsies with large numbers of lymphoid follicles offer a higher probability of detecting infected individuals (Wolfe et al. 2002). Biopsies with fewer follicles contain less, but still some, meaningful information. In seeking a method to account for this uncertainty, we developed a Bayesian model that simultaneously identified the probability that an individual is infected and the probability that a single follicle from an infected individual shows PrP CWD. Infection and detection probabilities were determined from numbers of test-positive and test-negative follicles found in tissue biopsies collected repeatedly from individual mule deer. After identifying the probability that a single follicle showed PrP CWD, we estimated the chances of producing a correct test result associated with a range of total follicle counts.

MATERIALS AND METHODS

Data collection

We collected samples in conjunction with a capture–mark–recapture study of free-ranging mule deer in north-central Colorado, US. Handling and sampling occurred during January 2010–2014. Two hundred ten adult (≥1.5 yr) female mule deer were captured by helicopter net gun and transferred to nearby processing locations. Individual deer were captured between one and five times, but only once during a single year. Five hundred nineteen biopsies were collected during this research.

Rectal-anal mucosa–associated lymphoid tissues (RMALT) were collected by methods described elsewhere (Wolfe et al. 2007; González et al. 2008) and CWD status was determined through immunohistochemistry (IHC) staining of RMALT (Spraker et al. 2002). The IHC analyses were completed at the Colorado State University Veterinary Diagnostics Laboratory (Fort Collins, Colorado, USA).

We estimated age by tooth wear patterns (Robinette et al. 1957). Genotype of the prion precursor (PRNP) gene was determined with the approach of Jewell et al. (2005) and confirmed by Sanger sequencing. Deer were fit with a mortality-sensing collar (Advanced Telemetry Systems, Isanti, Minnesota, USA). Field personnel determined weekly survival with the use of standard telemetry. Carcasses were sampled to obtain a postmortem test of CWD infection. The head and spinal column were collected from carcasses whenever possible. Field samples were transported to the Colorado State University Veterinary Diagnostic Laboratory for gross examination (as applicable), and IHC testing of tonsil, retropharyngeal lymph node, dorsal motor nucleus of the vagus nerve, or spinal column. All animals were handled in accordance with Colorado State University IACUC protocol 11-2758A.

Background on statistical methods

We developed a model that allowed CWD status to change with test occasion from susceptible to infected. Deer were either infected with CWD or susceptible at the time of the first disease test. Deer that entered as susceptible could become infected each year thereafter and, once infected, animals remained infected for the duration of study (or until they died). We allowed for false-negative diagnosis when a test result from an infected animal was negative. However, for simplicity we did not allow for a false-positive test
(wherein a susceptible animal was found positive) based on the high reported specificity of IHC.

We assumed that detection varied among individuals. PrP\textsuperscript{CWD} distribution in lymphoid follicles is thought to become more uniform over the disease course (Fox et al. 2006). Time since exposure and dose of exposure likely influence the proportion of follicles with PrP\textsuperscript{CWD} but the nature of our research did not allow us to know the timing or extent of exposure. Furthermore, we collected biopsies from deer annually and on as many as five test occasions. When deer were recaptured or there was visible scarring from previous tissue removal, biopsies were collected lateral to the optimal ventral location. Changes in location may have contributed to variation in the number of follicles collected and the proportion of follicles found with PrP\textsuperscript{CWD} in the same individual among years. Collecting biopsies may have induced the development of new isolated lymphoid follicles in the remaining rectal mucosa (Lorenz et al. 2003; Sipos et al. 2010), thereby affecting the number of follicles and proportion with PrP\textsuperscript{CWD} in subsequent biopsies.

In addition to potential sampling influences, prion disease progression is affected by polymorphisms in PRNP, the gene encoding the host's cellular prion protein (Ryon 2007). In mule deer, a nonsynonymous substitution of phenylalanine (F) for serine (S) at codon 225 (Jewell et al. 2005) appears to increase the time course of CWD in 225SF relative to 225SS genotypes, and delays deposition of PrP\textsuperscript{CWD} throughout the body (Fox et al. 2006). Furthermore, it appears that deer homozygous for phenylalanine (225FF) fail to exhibit IHC staining of tonsil or rectal tissue with infection (Wolfe et al. 2014). We intended to include genetic effects in our model, but too few infected deer of different PRNP genotypes were captured in our field study to evaluate this hypothesis fully (see Results).

For these reasons, we allowed age and additional unstructured individual-level variation to affect the proportion of follicles with PrP\textsuperscript{CWD} in an infected deer. We explain below how this conceptual model was represented as a Bayesian statistical model.

### Detailed statistical methods

We define $Z$ as an infectious status matrix. Elements of $Z$ are $z_{i,t}$ where $i$ is the individual deer and $t$ is the testing occasion, ‘year’ $i=1,\ldots,I$, $t=1,\ldots,T$. When an individual was not infected, $z_{i,t}=0$; when a deer was infected, $z_{i,t}=1$. The model for the initial test, $z_{i,1}$, is described below (equation 3). After the initial test, infection status at the current time $t$ is conditioned on infection status at the previous time $t-1$, where for $t=2,\ldots,T$,

$$[z_{i,t}|\psi, z_{i,t-1}]=\begin{cases} 1 & z_{i,t-1}=1 \\ \text{Bernoulli}(\psi) & z_{i,t-1}=0 \end{cases}$$

(1)

is the distribution of $z_{i,t}$ given the infection probability $\psi$ and $z_{i,t-1}$. Infection probabilities are assumed to be time invariant and similar between individuals. Our model assumes that an infected individual remains infected during the subsequent testing year and a susceptible individual becomes infected with probability $\psi$.

We define $Y$ as an observation matrix, where $y_{i,t}$ represents the observed number of follicles exhibiting PrP\textsuperscript{CWD} from individual $i$ during testing occasion $t$. We define the corresponding matrix, $J$, where $J_{i,t}$ is the total number of follicles obtained for individual $i$ at year $t$. False-positive test results were not believed to occur. Therefore, when $z_{i,t}=0$ then $y_{i,t}=0$. However, we may or may not have observed at least one positive follicle when an individual was infected, meaning when $z_{i,t}=1$ then $y_{i,t} \geq 0$. For deer $i$ at testing occasion $t$, the probability that an individual becomes infected is $\pi_{i,t}$, and

$$[y_{i,t}|\pi_{i,t}, J_{i,t}, z_{i,t}]=\begin{cases} 0 & z_{i,t}=0 \\ \text{Binomial}(J_{i,t}, \pi_{i,t}) & z_{i,t}=1 \end{cases}$$

(2)

The infectious status at time 1 depends on the observed infection value, where a false negative is possible. That is,

$$[z_{i,1}|\psi_0, y_{i,1}]=\begin{cases} 1 & y_{i,1} \geq 1 \\ \text{Bernoulli}(\psi_0) & y_{i,1}=0 \end{cases}$$

(3)

where $\psi_0$ is the probability that an individual developed disease prior to the initial testing occasion. There is an important distinction between $\psi$ in (1) and $\psi_0$ in (3); $\psi$ only captures the probability of infection during a single year, and $\psi_0$ is the population prevalence.

We incorporated individual effects on detection. We modeled $\pi_{i,t}$, the detection probability of individual $i$ on testing occasion $t$ in (2), with the age of deer $i$ at occasion $t$ used via a logistic model such that

$$\text{logit}(\pi_{i,t})=\beta_0+\beta_1 x_{i,t}+\varepsilon_{i,t}$$

(4)

Here $\varepsilon_{i,t}$ represents additional unstructured error where $\varepsilon_{i,t}$ is a normal random variable described by mean 0 and variance $\sigma^2$. Note that age was included as a categorical variable where deer were classified as 1–2 yr old or ≥3 yr old.
This model was fit to our data on mule deer with the use of the implementation described by Geremia (2014).

**Estimating the probability of a false-negative diagnosis**

We used the model described above to estimate the probability of false-negative diagnosis associated with age and numbers of follicles obtained in tissues. Numbers of test-positive follicles were derived with the use of Monte Carlo integration for deer 1–2 yr old and deer ≥3 yr old. For example, for a deer that was 1 yr old, the number of positive follicles \( y_{im} \) were generated from a binomial distribution with size \( J_{im} \) and probability equal to \( \text{invlogit}(b_0 + e) \), where the parameter \( b_0 \) is estimated via its posterior mean and \( e \) is generated from a normal distribution with mean 0 and (posterior estimate of the) variance \( \sigma^2 \). We varied \( J_{im} \), the total number of follicles found within tissue, between 1 and 20. Then the proportion of times when \( y_i \leq 0 \) provides an estimate of the probability of obtaining a false-negative test over all total follicle count levels for a young deer. The process was repeated for deer ≥3 yr old, except that the number of positive follicles was generated using a binomial probability equal to \( \text{invlogit}(b_0 + \beta_1 + e) \).

**RESULTS**

At least one isolated lymphoid follicle was obtained in 482 of 519 (93%) biopsies (mean = 8.6; standard deviation = 8.9; range = 0–72). Testing occasion and age affected the number of follicles obtained in a biopsy. These effects were interrelated because deer increase in age with each successive testing occasion. To examine the age effect, we evaluated follicle counts only using the first biopsy from each deer. Average follicle counts declined with age (linear model: \( P<0.001, R^2=0.14 \); Fig. 1). We examined the testing occasion effect by comparing follicle counts among same age deer at different testing occasions. Follicle counts for 2- and 3-yr-old deer declined with testing occasion (Fig. 2). Surprisingly, follicle counts remained otherwise constant, with some indication of increase with testing occasion among older deer (Fig. 2). In contrast to the strong decline in follicle count with age among the first samples collected from each deer (Fig. 1), overall follicle counts tended to increase with repeated biopsy despite increase in age of repeatedly tested individuals (Fig. 3).

We identified 20 deer that were PrP\textsubscript{CWD} positive at some point during our study (Fig. 4). Eleven deer were retested in years after first testing positive. Five of these deer were found negative in biopsies collected 1 or 2 yr after a positive test (Fig. 4). Only one of these individuals was tested a third time; that deer had a second positive test, providing assurance of a false-negative result (Fig. 4, deer M). Of the deer found negative after a positive test in a previous year, the total follicle counts for the PrP\textsubscript{CWD} negative tests were one follicle (Fig. 4, deer D and M), three follicles (deer T), four follicles (deer H), and 19 follicles (deer P).

Carcasses were recovered from 127 previously tested deer. Predation, scavenging, and accessibility complicated postmortem sampling. Consequently, we secured diagnostic postmortem samples from only 31 (24%) of these carcasses. Postmortem samples from six carcasses were PrP\textsubscript{CWD}...
positive in tonsil, retropharyngeal lymph node, dorsal motor nucleus of the vagus nerve, or spinal column. Five of these deer had been biopsy positive prior to death (including Fig. 4, deer D and T). The sixth deer shown as infected postmortem had PrP<sub>CWD</sub> in tonsil and retropharyngeal lymph node. This individual was biopsy negative 25 mo earlier (19 follicles detected) and again biopsy negative 13 mo earlier (two follicles detected) suggesting infection occurred sometime after initial testing and perhaps, prior to testing at 13 mo. Negative postmortem findings were confirmed for 25 other deer with negative biopsies on all prior testing.

We observed 15 deer that likely contracted CWD infection during our study. In each case, we detected at least one negative test where more than five follicles

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**Figure 2.** Numbers of lymphoid follicles detected in rectal-anal mucosa tissue biopsies of female mule deer (Odocoileus hemionus) that were annually captured and tested for chronic wasting disease in north-central Colorado, USA during 2010–14. Individual plots show follicle counts in same-age deer during subsequent testing occasions to illustrate the effect of repeated testing on follicle counts.
were examined providing >95% assurance of an accurate test (see below). Negative tests were followed by a positive test during a later year(s). We did not know the exact time of true exposure of these deer, but seven of the 15 deer that converted survived at least 365 d after diagnosis, five survived >500 d (Fig. 4, deer C, F, H, L, and P), and two survived >700 d (Fig. 4, deer H and P; see also Geremia 2014).

We detected 19 (of 136) biopsy-positive deer that were homozygous for serine (225SS) at codon 225, one (of 67; deer P) heterozygous for serine and phenylalanine (225SF), and zero (of four) homozygous for phenylalanine (225FF). These scant observations precluded fully evaluating PRNP effects on infection or detection, but we found preliminary evidence that the probability of an isolated lymphoid follicle testing positive in an infected deer was lower in 225SF deer: the percentage of follicles testing positive in deer that were known to be positive was 0.67 (SD=0.35) in 225SS and 0.50 (SD=0.71) in 225SF deer.

The probability that a given follicle being found positive for PrP\textsuperscript{CWD} in an infected individual was higher in younger deer (Fig. 5): 0.99 (0.94–0.99, equal-tailed 95% credible interval) for deer 1–2 yr old and 0.58 (0.18–0.90) for deer ≥3 yr old. PrP\textsuperscript{CWD}-positive deer tested more than one time exhibited additional variation in the chance of detecting a positive
follicle than described by age effects alone (Fig. 4, $\sigma=0.61, 0.20-2.24$). Some of this variation was accounted for by genotype. For example, removing the one 225SF deer showing infection (deer P) from analysis increased the probability that a given follicle was found positive for PrP CWD in older deer to 0.65 (0.30–0.90) and lowered the posterior estimate of $\sigma$ to 0.50 (0.01–1.80). Nonetheless, other individual-level factors beyond what we measured contributed to the CWD testing results we observed.

Given these findings, we determined the probability of a false-negative test associated with ages and follicle counts (Fig. 6). In 225SS mule deer 1–2 yr old, biopsies with at least one follicle had a >95% probability of correctly identifying an infected deer. In 225SS deer ≥3 yr old, biopsies required five follicles to have a 95% probability of detecting an infected deer and 10 follicles for a 99% detection probability.

**DISCUSSION**

Reliably detecting prion infection in mule deer requires some consideration of sample quality. Our findings resemble earlier work suggesting examination of at least nine lymphoid follicles in a tonsil biopsy might be necessary to determine CWD status in mule deer accurately (Wolfe et al. 2002). We found that examining five follicles in a rectal biopsy of 225SS mule deer, regardless of age, should ensure 95% probability of an accurate test; negative results were less conclusive for deer genotypes including phenylalanine (225SF, 225FF). Importantly, examining fewer follicles provided meaningful, but less certain information about the disease status of the individual. For example, fewer than five follicles were observed in 13 of 31 (42%) tests on animals that were confirmed PrP CWD negative postmortem. These less-conclusive live tests ensured 61% probability of the correct result when one follicle was obtained, and increased to 82% with two follicles, 91% with three, and 94% with four. Likewise, we encountered four apparent false-negative results in 225SS deer. In each case, we could not ensure a 95% accurate test based on deer
age and numbers of follicles in biopsies.

Rarely have individual animals infected with prion disease been repeatedly tested after a positive test. Instead, infected animals have generally been presumed to remain positive if retested because postmortem exams have confirmed their infection status (e.g., Wolfe et al. 2007; González et al. 2008). This belief appears well-founded based on evidence that prion diseases are progressive and that the proportion of positive lymphoid follicles increases over the course of infection (e.g., Fox et al. 2006). Given this well-established pattern, we were surprised that nearly half of the follow-up biopsies collected from deer that had already yielded a positive biopsy were negative.

Repeated biopsy of the rectal mucosa may have given rise to these false-negative tests. Isolated lymphoid follicles show dynamic properties, including de novo formation in adult animals (Lorenz et al. 2003). Gut-associated lymphoid tissue serves a variety of mucosal barrier defense functions, and isolated lymphoid follicles have been suggested to play a role in mucosal repair (Sipos et al. 2010). If the damage resulting from a biopsy stimulated new isolated lymphoid follicles to form in adjacent rectal mucosa, then the follicles available for subsequent sampling would be a mix of newer and older follicles. Because new follicles (≤12 mo old) would not have the same opportunity for prion accumulation as older follicles, their presence in nearby spans of mucosa could dilute or supplant the IHC-positive follicle pool in subsequent samples even as PrP\textsuperscript{CWD} accumulation progressed unabated in static lymphoid structures that remain undisturbed. This phenomenon could explain the static or declining proportion of positive follicles observed in biopsies from some infected individuals that were repeatedly sampled (Fig. 4) as well as the pattern of increasing follicle counts in repeatedly sampled individuals in the face of aging (Fig. 3). If isolated lymphoid follicle formation occurs in response to rectal mucosa biopsy, then repeated sampling could lower the likelihood of detecting infected animals, particularly in individuals genetically inclined toward more gradual disease progression. Alternatively, we implicitly assumed no laboratory errors occurred in the processing of biopsy samples. However, because three of the four false-negative cases came from the same year’s IHC accession, we cannot preclude the possibility of a systematic error somewhere in the course of testing.

The decline in the proportion of isolated lymphoid follicles showing PrP\textsuperscript{CWD} in older deer did not appear to be solely the result of repeated testing and associated disruption of tissue structure. Among eight deer that were biopsy positive on first testing, the proportion of follicles showing PrP\textsuperscript{CWD} in 1–2-yr-old deer was 100% (n=2), whereas proportions ranged from 27% to 100% (mean of proportions 63%) in ≥3-yr-old deer (n=6). We speculate that the higher proportion of positive follicles in young mule deer may result from greater activity in the immature lymphatic system or greater exposure because of close association with an infected dam or contaminated environment. Regardless of whether the foregoing observations were an artifact of small sample size, in the absence of repeated biopsy, age appeared to decrease ability to detect infection because fewer isolated lymphoid follicles were obtained in biopsies of older deer.

Every test sample is not the same; each individual exhibits unique variation, and the technique for estimating CWD infection that we developed here can account for some of these complications. Disease status becomes a probabilistic statement conditioned on the current test result, previous disease status, and infection and test sensitivity probabilities. Therefore, uncertainty in sampling becomes incorporated into the placement of individuals into discrete disease categories. This step forward allows us to make explicit probabilistic statements...
about whether an individual is infected and the chance that a test result is correct. With CWD, rather than conclude that an individual is not infected based on a test with few follicles or decide that the test was inconclusive, we can now state the probability that an individual is truly infected. Consequently, we can make conclusions that “a 90% chance exists that this deer is not infected, based on the results.”

Surveillance and containment programs for CWD benefit from an ability to diagnose animals correctly with the use of antemortem tests. Our model can easily be applied to surveillance on mule deer, facilitating use of all available samples regardless of total follicle counts. Probabilistic estimates of the infection status of each tested individual could then be used to provide 95% credible intervals of population prevalence that account for differences in test quality. Our model is robust to differences in population prevalence except when prevalence is low (e.g., <0.02%), because the detection and infection parameters become inestimable. When planning surveillance in areas where disease may not occur, we recommend assuming values for the test detection parameters to allow for estimation of population prevalence. Our approach also has application to CWD screening for transport of wild or captive deer or targeted culling efforts. Individuals could be identified that require additional testing to confirm disease status with desired levels of certainty, although our approach cannot account for misdiagnosing deer in early stages of infection when PrP(CWD) is undetectable (Wolfe et al. 2002, 2007). In light of our findings, further attention to the potential for repeated sampling to lower the probability of detecting infection via rectal mucosa biopsy appears warranted before such approaches are substituted for more conventional surveillance that relies on samples collected postmortem.

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LITERATURE CITED


Lachish S, Gopalaswamy AM, Knowles SCL, Sheldon BC. 2012. Site-occupancy modelling as a novel framework for assessing test sensitivity and estimating wildlife disease prevalence from...


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