PrP CWD in rectal lymphoid tissue of deer (Odocoileus spp.)

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The utility of rectal lymphoid tissue sampling for the diagnosis of chronic wasting disease (CWD) infections in mule deer (Odocoileus hemionus) and white-tailed deer (Odocoileus virginianus) was evaluated. CWD-associated prion protein (PrP CWD) deposits were observed in the rectal mucosa from 19 orally inoculated mule deer by 381 days post-inoculation (p.i.); similarly, 45 out of 50 naturally infected mule deer had PrP CWD in their rectal mucosa. In orally inoculated white-tailed deer, the presence of glycine (G) or serine (S) at codon 96 of the native PrP (denoted 96GG, 96GS or 96SS) appeared to influence the temporal patterns of PrP CWD deposition: nine out of 11 infected 96GG individuals had PrP CWD in their rectal mucosa by 342 days p.i., whereas only three out of seven infected 96GS individuals had PrP CWD in their rectal mucosa by 381 days p.i. and none of three 96SS individuals had PrP CWD in their rectal mucosa by 751 days p.i. These findings support further evaluation of rectal mucosa sampling in CWD surveillance.
To assess the utility of RMALT sampling for diagnosis of CWD infections in deer, we used 19 captive mule deer and 21 captive white-tailed deer that had been experimentally infected at about 6 months of age by oral inoculation with 1 g of conspecific, undiluted, infectious brain tissue pool (based on previous analyses, inoculum pools contained about 3 or 6 µg PrP<sub>CWD</sub> g<sup>−1</sup>; Sigurdson et al., 1999; Raymond et al., 2000); these inoculated deer were part of an ongoing study of agent shedding patterns (Colorado Division of Wildlife Animal Care and Use file 7–2004). To facilitate sampling, we anaesthetized deer with xylaine (50–100 mg) and ketamine (40–100 mg) and applied a topical analgesic cream (2.5% lidocaine and prilocaine, Fougara Cream; E. Fougara and Co.) to the distal rectal mucosa. Sampling methods were adapted from those used by L. González and M. P. Dagleish in sheep (González et al., 2005; and unpublished data). About 10 min after applying the local anaesthetic cream, we exposed the rectal mucosal border by manually exteriorizing the anal mucosa or isolating it using a home-made speculum (M. P. Dagleish, unpublished data). Using Brown–Adson forceps, the mucosa was lifted from a depression between the rectal columns in the 0.8–1 cm immediately rostral to the transition between the anal orifice and the mucosa (mucocutaneous junction). A small piece (5–6 mm diameter) of the elevated mucosa was cut with fine-point scissors or rectal biopsy forceps. Bleeding was controlled with direct pressure and Gel Foam (Pharmacia & Upjohn Company) applied as needed. Additional topical analgesic cream was applied directly to the biopsy sites. We collected rectal biopsies at 253 days post-inoculation (p.i.) from all deer and at 299, 342, 381, 477, 552, 661 and 751 days p.i. when previous results were negative or samples were inadequate. For comparison, we also collected tonsil biopsies at 253 days p.i. using methods described previously (Wolfe et al., 2002) and again at 342 and 477 days p.i. in individuals with negative biopsies at the preceding sampling. Tissue samples were placed in 10% neutral buffered formalin and submitted for evaluation for PrP<sub>CWD</sub> by immunohistochemistry using methods established for tonsil biopsy (Miller & Williams, 2002; Wolfe et al., 2002). We also sampled 45 naturally exposed and 20 unexposed adult (≥1.5 years old) mule deer using the methods described above. In addition to RMALT sampling, we collected blood or tissue, extracted the DNA and determined the PrP genotype using established methods (O’Rourke et al., 2004; Jewell et al., 2005).

All 19 experimentally infected mule deer had PrP<sub>CWD</sub> deposits in tonsil biopsies (termed ‘tonsil positive’) when sampled at 253 days p.i. Concurrently, we observed PrP<sub>CWD</sub> deposits in rectal mucosal biopsies (termed ‘RMALT positive’) from 15 out of 17 mule deer (88%) where adequate samples were collected (Fig. 1); two samples were insufficient. By 381 days p.i., all 19 mule deer were RMALT positive.

Twenty-six of the 45 naturally exposed mule deer examined were tonsil positive, but RMALT samples from two of these were insufficient for evaluation. Of the 24 tonsil-positive deer with usable RMALT samples, 20 (83%) also were RMALT positive. All 20 unexposed deer were RMALT negative.

Of the 21 captive, experimentally infected white-tailed deer, 16 were tonsil positive at 253 days p.i. and nine (56%) of those were also RMALT positive. However, the PrP genotype [encoding combinations of glycine (G) and serine (S) at codon 96, denoted 96GG, 96GS or 96SS; O’Rourke et al., 2004] appeared to influence the patterns of PrP<sub>CWD</sub> deposition in tonsil and RMALT of white-tailed deer (Table 1). Nine out of ten 96GG white-tailed deer were tonsil positive when sampled at 253 days p.i. and eight (89%) of the nine tonsil-positive deer were also RMALT positive; all ten were tonsil positive by 342 days p.i. and nine (90%) of these were also RMALT positive. In contrast, seven out of eight 96GS white-tailed deer were tonsil positive when sampled at 253 days p.i., but only one (14%) of the seven tonsil-positive deer was RMALT positive; all eight were tonsil positive by 342 days p.i., but only four (50%) of these were RMALT positive by 381 days p.i. By 751 days p.i., five of the six 96GS white-tailed deer (83%) with usable samples were RMALT positive. The three 96SS white-tailed deer were tonsil and RMALT negative at 253 and 342 days p.i. (Table 1). At 477 days p.i., all three 96SS white-tailed deer became weakly tonsil positive, but they all remained RMALT negative up to 751 days p.i. As all mule deer in our inoculation study were homozygous for S at codon 225, we did not have an opportunity to explore whether PrP genotype [encoding S or phenylalanine (F)] might have any influence on the patterns of PrP<sub>CWD</sub> deposition in this species; however, two of the four naturally exposed deer that were tonsil positive but RMALT negative were SF at codon 225. In light of the apparent effects of PrP genotype on other aspects of CWD pathogenesis (Jewell et al., 2005; Fox et al., 2006), influences similar to those seen here in white-tailed deer would not be surprising.

As with tonsil biopsies (Wolfe et al., 2002), sample quality should be considered when interpreting data from RMALT biopsies to diagnose CWD in individual deer. Overall, 138 out of 161 RMALT biopsies (86%) had observable lymphoid follicles; based on experiences with tonsil biopsy (Wolfe et al., 2002), we anticipate that sampling consistency will probably improve with experience and minor technique improvements. Overall sampling success was similar for the two deer species: 58 out of 69 rectal biopsies (84%) from white-tailed deer and 80 out of 92 rectal biopsies (87%) from mule deer contained follicles. Individual variation may have contributed to problems with sample quality: six of the 15 ‘insufficient’ mule deer biopsies came from three animals. Although not studied here, age could further influence the abundance, location or appearance of true lymphoid follicles in deer: in the white-tailed deer that were serially sampled, eight out of 11 insufficient samples were from the later sampling periods, suggesting that age or repeated sampling may affect RMALT biopsy quality.
Potential variation in sample quality should be less of a consideration with post-mortem sampling, because the distal rectum can be opened for sampling and larger amounts of tissue can be collected for analysis (Espenes et al., 2003, 2006; González et al., 2005, 2006). In addition to examining biopsies from live deer, we also collected and examined rectal mucosa samples post-mortem from 48 naturally exposed mule deer to examine the correlation between PrP\textsubscript{CWD} deposits in medial retropharyngeal lymph nodes and lymphoid follicles in the rectal mucosa; 26 of these deer had PrP\textsubscript{CWD} deposits in retropharyngeal lymph node tissue whilst 22 did not. We saw agreement between immunohistochemistry of rectal mucosa and retropharyngeal lymph node in 47 of the 48 cases (κ=0.96; 95% CI 0.88–0.99); no PrP\textsubscript{CWD} was detected in the rectal mucosa of one infected deer. Moreover, when we screened independent rectal mucosa samples from 28 of these deer using an ELISA previously validated for use in CWD surveillance (Hibler et al., 2003), PrP\textsubscript{CWD} was detected in 12 of the 14 infected deer (absorbance values ≥0.201). Based on observations from microscopic examination of immunohistochemistry samples, failure to detect two of the 14 infected deer by ELISA seemed to be more likely to be due to sample inadequacy than to failure of the ELISA to detect PrP\textsubscript{CWD} in these samples.

Our findings support the further evaluation of RMALT sampling as an alternative to sampling lymphatic tissues of the head and neck in CWD surveillance programmes. For screening of either free-ranging or captive deer populations to detect the presence of CWD, our data show that RMALT sampling should detect a high proportion of infected individuals, particularly those in the later stages of infection; technical improvements with increased experience should also lead to better results in early infection. However, the use of RMALT sampling in CWD surveillance may be limited by the ability to acquire a sample with adequate follicles for evaluation. Additional data from naturally infected mule deer and white-tailed deer would be useful for better estimation of the sensitivity of RMALT sampling compared with cranial lymph node sampling; given such data, sample sizes or prevalence estimates could be adjusted to accommodate the somewhat lower sensitivity anticipated from our data. When the total cost of sampling and disposal from the head and neck are considered, rectal mucosal sampling testing is likely to be more economical and efficient than the existing testing system. Even taking into account the possible need to use greater numbers of rectal mucosal tests to achieve the same sensitivity as testing tissues of the head, the greater ease of sampling, cost of disposal and its suitability for rapid test analysis (L. González et al., unpublished data) suggest that
rectal mucosal testing offers a more economical and efficient approach for large-scale surveillance schemes. As a tool for assessing individual infection status for movement or management purposes, RMALT biopsy may not be as reliable as tonsil biopsy in deer, as PrPCWD appears to be deposited somewhat later in lymphoid tissues of the rectal mucosa compared with tonsil follicles; based on our data from white-tailed deer, relevant PrP genotypes probably need to be considered for both deer species in interpreting negative results. Rectal mucosal biopsy could be useful as a screening tool in captive herds or free-ranging conditions where anaesthetizing individuals for handling is either undesirable or unnecessary. However, our data show that tonsil biopsy or tonsil biopsy in addition to rectal biopsy is still preferred for reliable detection of CWD infection in individual deer.

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**References**


**Table 1. PrP codon expression and the time frames for PrP CWD deposition in tonsil and rectal biopsy samples from orally inoculated white-tailed deer**

<table>
<thead>
<tr>
<th>Animal</th>
<th>PrP codon</th>
<th>Days post-inoculation</th>
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<tr>
<td></td>
<td>95  96  116</td>
<td>253  342  381  477  552  661  751</td>
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<tr>
<td>Tonsil</td>
<td>RMALT</td>
<td>Tonsil</td>
</tr>
<tr>
<td>N104</td>
<td>QQ</td>
<td>GG</td>
</tr>
<tr>
<td>K204</td>
<td>QQ</td>
<td>GG</td>
</tr>
<tr>
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<td>GG</td>
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<td>GG</td>
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<td>QQ</td>
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*Results reported as positive (+), negative (−) or insufficient follicles (ISF) for PrP CWD deposition in the respective tissue. Deer that died or were euthanized due to clinical CWD (c) or other causes (o) are indicated.

*Amino acids: glutamine (Q), glycine (G), alanine (A), serine (S), histidine (H).
samples from sheep with experimental scrapie. J Comp Pathol 134, 115–125.


