Use of Parotid Lymph Node Tissues For Chronic Wasting Disease Surveillance in Hunter-Killed and Live White-Tailed Deer

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Abstract:

Most state wildlife agencies have enacted surveillance efforts to determine if chronic wasting disease (CWD) is present in North American deer (Odocoileus sp.). Brain stem and retropharyngeal lymph node (RLN) samples are commonly collected from sick or hunter-killed deer. Testing live deer for CWD has been accomplished using tonsillar biopsy tissues. Parotid lymph nodes (PLN) offer another potential alternative for CWD testing during hunter-killed deer surveillance and as an antemortem test. We describe PLN collection procedures from hunter-killed and captive white-tailed deer and discuss the merits of PLN collections for CWD surveillance. Our evaluation took place in three stages: we collected PLN tissue samples 1) at check stations during the 2004 Missouri firearms deer season; 2) from subsets of hunter-killed and road-killed deer to identify the location of the PLN and evaluate biopsy tools to refine sampling techniques that could be used on live deer; and 3) from 10 captive white-tailed deer. Collections of PLNs at check stations were efficient and

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required less time than on-site collections of RLN or obex brain tissue or tissue collections at a centralized location, and all PLN samples were usable. During stage two, we developed a template to find the location of the PLN and observed that a scalpel/scalpel combination and scalpel/punch combination provided the most usable samples. After immobilization, it took on average 14.5 minutes to collect a PLN sample from live animals. Of the 28 PLN tissue samples obtained from live deer, all were usable. Advantages of collecting PLN samples included: 1) less time resulting in less agency personnel investment and reduced inconvenience to hunters; 2) ease of locating and collecting parotids from hunter-killed deer, which should result in fewer erroneous tissues collected; and 3) overall cost savings; our estimated cost per collected sample was $33–62 for live animals and $12 for hunter-killed deer samples (including labor). Because of these factors, agencies conducting large-scale CWD surveillance efforts should consider PLN collections to reduce costs and increase the number of samples that can be collected with limited personnel. For live animals, collection of PLN samples is a viable alternative. More studies are needed to explore the staging of CWD progression to PLNs and other superficial nodes

Key Words: *antemortem test, chronic wasting disease, Odocoileus virginianus, parotid lymph node, surveillance, white-tailed deer*

1. Introduction

Chronic wasting disease (CWD), an infectious neurological disease of North American deer (*Odocoileus sp.*) and Rocky Mountain elk (*Cervus elaphus nelsoni*), belongs to a family of infectious diseases known as transmissible spongiform encephalopathies (Williams and Young 1980, 1982, 1992). Williams et al. (2002) linked the distribution of CWD in farmed elk to market-driven movements of infected elk among cervid facilities. Recent discoveries of CWD in captive white-tailed deer (*Odocoileus virginianus*) in Wisconsin and New York suggest interstate movements of captive white-tailed deer increase the risk of introducing CWD (Samuel et al., 2003). The natural expansion of CWD in free-ranging animals is slow (Williams et al., 2002); however, individual cervid movements can be significant (e.g., Petersburg et al., 2000, Mackie et al., 2003) and natural geographic expansion through cervid populations occurs.

Early detection of CWD is essential to its management and most states have enacted surveillance efforts to determine if the disease is present. Chronic wasting disease surveillance most often involves targeted surveillance (Williams et al., 2002), geographically stratified random testing (Conner et al., 2000), or both. Despite reported inefficiencies associated with hunter-killed deer surveillance (Diefenbach et al., 2004) the technique has detected CWD foci in a number of states and represents a reasonable alternative for many state agencies. Brain stem (obex of the medulla oblongata), and retropharyngeal lymph node (RLN) samples from sick or hunter-killed deer are tested using an immunohistochemical (IHC) test for proteinase-resistant prion proteins (Miller and Williams, 2002). Prions were especially high in RLNs and mesenteric-portal lymph nodes, which originally justified their use (Hadlow et al., 1982). Testing live deer for CWD has been accomplished with collection of tonsillar biopsy tissues (Wild et al., 2002; Wolfe et al., 2002, 2004). Regardless of the technique used, live deer detection protocols require that a substantial number of animals be tested if the desired goal is to detect the disease at low prevalence rates.

Parotid lymph nodes (PLN) offer another potential alternative for CWD testing hunter-killed deer for CWD and as an antemortem test for white-tailed deer. The PLN is located on the lateral surface of the head just ventral to the temporomandibular joint. It receives drainage from many external and internal structures of the head, including portions of the nasal and oral cavities, eyes and ears; the skin; several bones; and most of the muscles. It drains into the lateral retropharyngeal lymph node (Saar and Getty, 1975; Schummer et al., 1981). Parotids can reveal CWD infections at least as early as obex brain tissues (Beth Williams, Wyoming State Veterinary Laboratory, personal communication). Collections of obex brain tissues and RLNs can be labor-intensive but PLNs might be more easily obtained from hunter-killed deer.

We describe PLN collection procedures from hunter-killed and captive white-tailed deer and discuss the merits of PLN collections for CWD surveillance and as an antemortem test. Our objective was to determine the feasibility of PLN collection from hunter-killed deer and assess whether PLN sampling was possible from live animals.

2. Study Area and Methods

We conducted field evaluations of the technique at the Dutch Mill, Mid-Missouri Taxidermy, and Sinclair deer check stations in Gasconade, Randolph, and Howard counties, Missouri, respectively. We measured distances from deer facial features to PLNs and collected samples from hunter-harvested deer at the Resource Science Center, located in Columbia, Missouri.

Live-deer evaluations were conducted at the Olympia, Washington field station of the United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center (NWRC). The NWRC maintains a captive herd of white-tailed deer at the facility for research purposes.

a. Field evaluations of PLN collections

To evaluate and compare the costs and efficiency of PLN collections from hunter-killed deer we collected PLN tissue samples during the Missouri firearms deer season, 13-23 November 2004. We compared these data to collection procedures, time requirements, and costs for RLN and obex tissue collections that we previously reported (Beringer et al., 2003). Three biologists collected PLN samples and 51 different biologists collected RLN samples. All individuals received 4 hrs of field training.

Collection procedures for PLN tissues from hunter-killed deer were as follows: we positioned the deer so one side of the head was exposed. Using a scalpel, we made a 2-cm-deep, 5-7-cm-long cut through the skin and subcutaneous tissues in the area of the PLN. To determine where to make the incision, we used our thumb to locate the notch on the ventral surface of the zygomatic arch at the mandibular fossa (we termed this the "zygomatic notch; Zn"). In most deer a shallow groove can be palpated running between the "notch" and the ramus of the mandible. Holding the thumb against the notch, we cut from just behind our thumb to 5–7 cm ventrally along the groove. This procedure almost always transected the PLN; if not, we explored the tissue to either side of the cut to locate the node (Figure 1).

After locating the PLN, we used a forceps and scalpel to remove a 5- x 5-mm piece of node tissue and placed it into a formalin-filled vial. We recorded time required to remove PLNs starting when a tissue collector first positioned a deer until the tissue was placed into the vial.

Figure 1. Parotid lymph node (PLN) removal from an adult male white-tailed deer (*Odocoileus virginianus*) at Missouri hunter check station. The scalpel blade points to transected PLN.

b. Measuring distances to PLNs relative to facial landmarks in hunter-harvested deer

Collection of PLNs from live deer requires that tissue collectors be able to accurately locate the PLNs prior to surgical removal to minimize the technique's invasiveness and to ensure that at least 6 follicles are obtained for IHC analysis. To enhance our ability to accurately locate incision sites for PLN sampling from live deer we measured the distance to PLNs relative to identifiable skull or facial landmarks using a second set of hunter-killed deer (Figure 2). In domestic ruminants there is some individual variation in size, shape, number, and location of PLNs (Schummer et al., 1981); we were interested in determining whether the PLN location in deer is

sufficiently consistent to accurately place a biopsy tool without the larger incision required to see the node. On this set of hunter-killed deer we measured both sides of each head except those on which landmarks had been damaged by bullet wounds or processing. We used several types of metric-scale scientific and architectural rulers, protractors, and calipers to make the measurements. We dissected to the PLN with scalpel and forceps. Measurements using landmarks that were difficult to consistently palpate through the skin or otherwise proved unreliable were discontinued.

We also identified nearby sensitive structures that could complicate removal of the PLN in a live animal: the transverse facial artery and vein, the auriculotemporal and dorsal buccal branches of the facial nerve, and the parotid salivary gland and duct (Figure 3). The vessels and nerves in the area ran medial to the PLN and could be seen emerging from beneath its periphery. The parotid salivary gland lies just caudal to the PLN and its rostral edge overlaid the caudal surface of the node in some deer; its duct left the ventromedial aspect of the salivary gland and ran ventrally along the angular process of the mandibular ramus and so was not within the PLN surgical field.

Margin of error for live animal evaluations was small so we developed a template that could be positioned on the deer's head to identify the location of the PLN (Figure 4). Because of differences between males and females and age of the animal (i.e., fawn [1 year of age], yearling [>1 year and < 2 years of age], and adult [2 years of age]) we report mean measurements and their standard deviation separately by age and gender. To develop the template we considered the measurements mentioned above and their consistency as measured by their standard deviation. We viewed those measurements that were easy to obtain and most consistent as potentially good indicators of PLN location. From these data we developed 6 templates (by sex and age group combinations) that could be used to locate the PLN during live-animal sampling. We also developed templates to facilitate efficient collection of PLNs from hunter-killed deer during the initial stages of sampling when people were less familiar with the location of the PLN (see below).

Figure 2. Mapping the location of the parotid lymph node (PLN) on a white-tailed deer head. Be = back of eye; Zn = zygomatic notch; Mr = ramus of the mandible; and Fe = front of the eye; En = ear notch; and Vcaw = ventrolateral corner of the atlas wing.

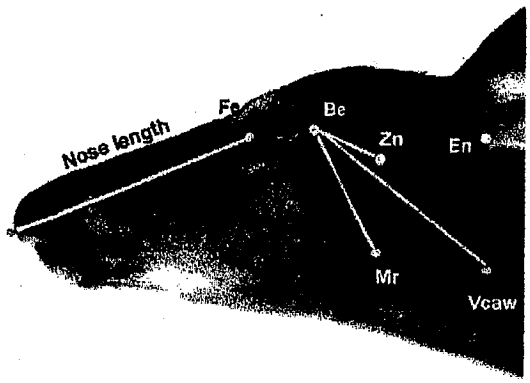


Figure 3. The left parotid lymph node (PLN) in a white-tailed deer with skin and superficial tissues removed. 1 = transverse facial artery and vein; 2 and 3 = auriculotemporal branch and dorsal buccal branch of facial nerve; 4 = parotid salivary gland, separated from PLN; and Zn = location of zygomatic notch.

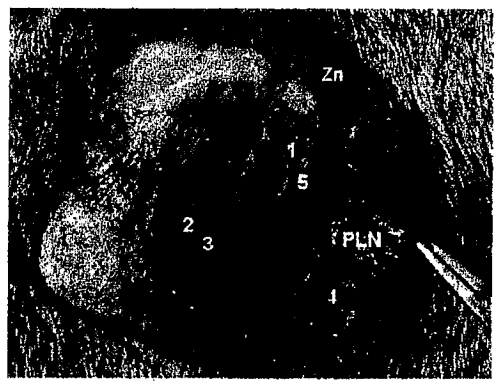
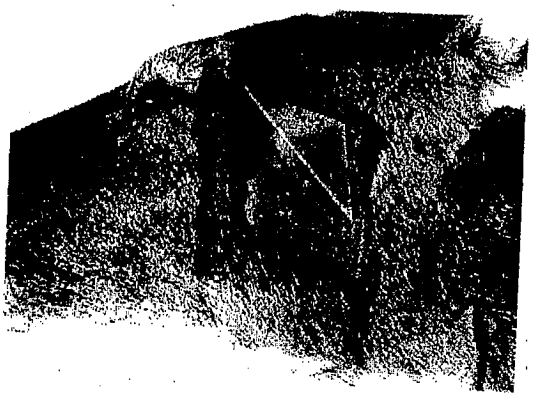


Figure 4. The parotid lymph node template positioned on adult white-tailed deer head. One corner of the template was positioned on back of the eye (Be) and another aligned with the zygomatic notch (Zn). The location of the parotid lymph node is indicated by the arrow labeled "Parotid" on the template.



c. Evaluating techniques for collecting PLN samples using hunter-killed deer

We considered appropriate sample collection options for the live-animal PLN collection technique using a third set of hunter-killed deer. During this phase of the project we attempted to mimic live-animal collections (e.g., we limited incision length). To collect PLN tissues we used electric clippers to shave an approximately 12-cm-sq area on each side of the head; we aligned the appropriate template with the back of the eye and Zn and marked the predicted location of the PLN (Figure 4). We evaluated the following tool combinations for making the skin incision and collecting PLN tissues: biopsy punch/biopsy punch, scalpel/biopsy punch, and scalpel/scalpel. We measured the proportion of usable PLN samples for each combination. We fixed tissue samples in a 10% buffered formalin solution and sent them to the Wyoming State Veterinary Laboratory to determine if the tissues had adequate follicles for IHC analysis. We considered a usable sample to have at least 6 lymphoid follicles.

d. Live-animal PLN collection

A veterinarian collected biopsies of PLNs on 20-21 January 2005. We fasted the deer for 12 hrs in order to reduce the risk of bloat, regurgitation or aspiration of regurgitated material during anesthesia (Lin and Pugh, 2002; Allen and Borkowski, 1999). We then herded individuals into a handling system with a squeeze chute, where they were restrained, blindfolded, and sedated. We immobilized deer with tiletamine hydrochloride (HCl) –zolazepam HCl (Telazol®, Fort Dodge Animal Health, Fort Dodge, Ia.), xylazine HCl (Rompun®, Bayer Corporation, Shawnee Mission, Kans.) and ketamine HCl (Ketaset®, Fort Dodge Animal Health, Fort Dodge, Ia.) in one of 3 drug combinations: Telazol 4.4 mg/kg + xylazine 2.2 mg/kg IM, ketamine 4 mg/kg + xylazine 3 mg/kg, or xylazine 3 mg/kg IM. We tailored drug combinations to individual deer based on weight. We transferred deer to a table and placed them in sternal or right lateral recumbency once they were immobile.

We prepared the incision site by shaving an approximately 8-cm x 8-cm area, performed a standard scrub with chlorhexadine (The Butler Company, Columbus, Oh.) and alcohol, and applied a povidone-iodine solution (Betadine®, Purdue Frederick, Stamford, Conn.). Initial incisions were 2.5 cm long and made with #22 scalpel blades; they were centered over the PLN location as determined from our previous experience with dead deer. If necessary, we enlarged incisions (max. 5 cm) as we located and dissected to the PLN. We collected the biopsy with either a #15 scalpel (wedge biopsy) or a 5-mm dermal biopsy punch, severing the base of the plug with a scalpel; we took 2 samples per node, with an attempt to obtain one from each pole. We then flushed incisions with sterile saline. We closed incisions subcutaneously (if deeper than 0.5 cm and longer than 2.5 cm) with an absorbable suture and placed 1–2 absorbable subcuticular sutures. Additionally, on the second day we applied n-Butyl cyanoacrylate skin glue (Vetbond®, 3M Animal Care Products, St. Paul, Minn.) over the site except for the ventral 0.5 cm to allow for drainage.

To reverse immobilization we injected deer with 2 mg/kg tolazoline HCl (Tolazine™, Lloyd Laboratories, Shenandoah, IA), by administering ½ IV and ½ IM. Finally, as a prophylactic antibiotic, each deer received 6.6 mg CE/kg of ceftiofur (Excede®, Pfizer Inc., New York, NY) injected subcutaneously into the ear before the animal was carried to a stall for recovery. Staff monitored deer for post-operative complications during the following 2 weeks and observed them for signs of capture myopathy for 30 days. All procedures with

live deer were approved by the NWRC's Institutional Animal Care and Use Committee.

3. Results

a. Field evaluations on PLN collections

Collections of PLNs at check stations from hunter-harvested deer were efficient and required less time than on-site collections of RLN or obex brain tissues or tissue collections at a centralized location. Times to remove PLN tissues and place in formalin for Dutch Mill, Mid-Missouri Taxidermy, and Sinclair check stations averaged 35.2 sec (SD = 14.9, range = 19 – 80, n = 35), 28 sec (SD = 18.4, range = 8 – 110, n = 32) and 16.8 sec (SD = 5.4, range = 7 – 29, n = 62), respectively. All PLNs collected at check stations were usable samples (i.e., had at least 6 follicles). On-site RLN collections averaged 121 sec (SD = 56.2, range = 64 – 375, n = 81) and previously reported tissue collections at a centralized location (Beringer et al., 2003) took over 350 sec per sample. Our state-wide RLN collection effort averaged 97% usable samples with most unusable samples consisting of salivary gland or skeletal muscle. Equipment start-up costs for collecting PLN and RLN tissues were similar. Also, because of the location of PLNs they were unaffected by neck-shot deer and were unlikely to be confused with other tissues.

The increased collection efficiency of PLNs resulted in labor time savings and was more convenient for hunters. In our setting we were able to collect PLN tissues from even high-volume check stations (i.e., up to 400 deer per day) with 2 people, whereas we used 4 persons per check station to collect RLNs (Beringer et al., 2003). Missouri check stations are busiest on the opening weekend of firearms season and during the 2-hr period following sundown. Surveillance efforts are geared to handle these high-volume days and efficient tissue collections are important. We collected approximately 10 RLN and 20 PLN samples per scalpel blade.

b. Measuring distances to PLNs relative to facial landmarks in hunter-harvested deer

We measured distances to PLNs relative to facial landmarks on 222 hunter-killed and road-killed deer, including 123 adults, 91 yearlings, and 8 fawns; 136 were female and 86 were male (Table 1). Of the 17 different measurements, 3 had low standard deviations and were used to develop our template. These included Zn-P, Angle Zn-P to Be-Zn, and Be-p P/Be-Mr (Table 1).

c. Evaluating techniques for collecting PLN samples using hunter-killed deer

We collected 61 PLN samples from hunter-killed deer using our template and surgical techniques with combinations of a biopsy punch and scalpel. Of those, we located the PLN 95% (58/61) of the time based on the template developed from Table 1. All samples (n = 22) collected with a scalpel/scalpel combination were usable (i.e., parotid cortex with at least 6 follicles) while 15 of 16 or 93.7% were usable when collected with the scalpel/biopsy punch combination. When using the punch for the initial incision and for collecting the PLN only 8 of 23 samples or 34.8% collected were usable. Scalpel cuts facilitated locating the PLN after the initial incision. The biopsy punches we used were too small to determine whether we had collected a portion of PLN. The biopsy punch was quick and less invasive than scalpel cuts, but the low proportion of usable samples collected with biopsy punches made the technique inefficient.

d. Live-animal PLN collection

We performed biopsies on 10 deer (6 male, 4 female; ages 0.5 – 4.5 yrs); the left PLN was sampled on 8 deer and right PLN on 2 deer. We obtained 2 tissue samples per node. It took on average 14.5 minutes (SD = 3.59, n = 10) to collect PLN samples once animals were sedated. We obtained at least 1 usable sample from all deer. Even though we had experience finding PLNs from the dead deer we still collected some pieces of other tissues along with the PLN tissue in 2 deer; one included a piece of salivary gland and the other a fragment of muscle. All samples yielded a sufficient number of follicles (6) for an official test. Our PLN samples averaged 64 follicles (SD = 43.4, range = 149, n = 10). The sample obtained with a biopsy punch contained 26 follicles.

We had no serious complications from the anesthesia or surgery. There was no significant hemorrhaging, but minor bleeding increased the time required to locate the PLN. Bleeding did not, however, present a direct danger to the animal; most bleeding was from small capillaries that crossed the incision line which coagulated by the end of the procedure, or were clamped and then ligated during

wound closure. Branches of the facial artery, vein and nerve were seen near the incision site in some deer and were easily avoided. We encountered the parotid salivary gland in all deer, and dissected beneath it where necessary to expose the PLN. Most PLNs were about 1 cm diameter. One year after surgery, no deer had died as a result of the biopsies.

Expenses for our live-animal tests included anesthesia, standard surgical supplies and disposable instruments. Anesthetic cost was the main variable expense and ranged from approximately $7 to 28 per animal, with ketamine + xylazine being lowest and Telazol + xylazine the highest. Fixed costs included such standard surgical items as scrub solutions, sponges, instruments, sutures, skin glue and antibiotics; they totaled $14 per animal. Although we used a separate pack of reusable forceps and needle-holder for each deer in this captive research herd, we recommend using only disposable instruments in practice applications in order to reduce the possibility of prion transfer among animals; these would cost $8.50 per animal. The total cost per live deer was $33–62 and $12 for hunter-killed deer samples (including labor). The range in cost estimates accounts for variation in size of different deer, corresponding differences in amounts of drugs used, and variation in length of time required for procedure. Initial start-up costs, to purchase all instruments, surgical supplies and anesthetic agents would total $215 if using disposable instruments or $249 if purchasing reusable instruments.

Table 1. Summary of measurements (mean and standard deviation) used to develop a template to identify the location of the parotid lymph node (PLN).

	Females			Males		
Measurement	Fawn	Yearling	Adults	Fawn	Yearling	Adults
Zn-P[1]	1.86 (0.39)	1.96 (0.28)	2.05 (0.38)	1.79 (0.42)	2.09 (0.98)	2.01 (0.44)
Angle Zn-P to Be-Zn[2]	90 (6.02)	100 (7.39)	98 (8.04)	96 (3.54)	99 (9.41)	102 (7.64)
Be-p P/Be-Mr[3]	4.23 (0.54)	4.85 (0.47)	5.11 (0.61)	4.76 (0.38)	5.34 (0.52)	5.25 (0.63)

[1] Distance in cm from Zn ("Zygomatic notch": notch on ventral surface of Zygomatic arch at the mandibular fossa) to center of PLN.

[2] Angle (^{0})between Zn-P and Be-Zn (Back of eye; measurement point taken on caudal side of orbital bone at the lateral canthus, at the suture between the zygomatic process of the frontal bone and the frontal process of the zygomatic bone), to nearest degree.

[3] Distance in cm as measured along line Be-Mr (Back of eye to Mandibular ramus; measurement point taken on furthest dorsal and caudal edge of angular process of mandibular ramus); distance is taken from Be to where the perpendicular from En (ear notch) intersects Be-Mr with the head maximally extended so it is nearly parallel with the neck.

4. Discussion

Advantages of collecting PLN samples included: 1) less time resulted in less agency personnel time investment and reduced inconvenience to hunters; 2) ease of locating and collecting parotids from hunter-killed deer should result in fewer erroneous tissues collected; and 3) overall cost savings; estimated cost per collected sample from live deer was $33–62 and $12 for each hunter-killed deer sample (including labor). Because of these factors, agencies conducting large-scale CWD surveillance efforts might consider PLN collections to reduce costs and increase the number of samples that can be collected. Larger sample sizes would increase confidence that CWD is detected at low prevalence.

The utility of PLNs for detecting CWD is also dependent upon when PLNs reveal infection relative to other lymph tissues. The few studies that have evaluated the presence of CWD prions in superficial nodes had small sample sizes and observed considerable variation in rates of infectivity. Studies are needed to explore the staging of CWD progression to PLNs and other superficial nodes. Spraker et al., (2002) evaluated PLNs and other tissues in mule deer (*Odocoileus hemonius*) and found PLNs from infected dead wild and captive deer stained positive in 79% (n = 14) and 67% (n = 9) of cases, respectively; similar research with adequate samples should be conducted on white-tailed deer.

The PLN was an accessible tissue for obtaining samples from live animals. The amount of time required for collection was extended primarily due to anesthetic induction, clipping during surgical preparation, and minor hemorrhaging obscuring vision of the node. When performing surgery on multiple animals, delays can be reduced by beginning preparatory clipping in the squeeze chute as soon as the deer is sufficiently sedated. Also, administering anesthetic to a 2nd animal while the procedure is underway in the first would increase surgical efficiency. We recommend using a formulation of lidocaine that includes epinephrine to reduce hemorrhage at the site in order to facilitate identifying and biopsying the node. As field conditions can make aseptic techniques difficult and as deer hair sheds easily, use of an adhesive drape is suggested. We recommend practicing on dead deer prior to performing the procedure on live animals. The PLN area could be located with a template and marked with a surgical marker during preparation to facilitate correct placement of the incision on live deer.

In addition to time required to obtain the sample, operator experience required, and costs, the likelihood of obtaining suitable tissue for CWD analysis, and degree of PrPCWD accumulation in the tissue during pre-clinical infection are important considerations. Previous studies of tonsillar biopsy in preclinical deer showed varied effectiveness of collecting adequate follicles, with a significant proportion of samples containing no or few follicles even when done by experienced persons (Wild et al., 2002; Wolfe et al., 2002, 2004). In their initial development of tonsillar biopsy in mule deer and white-tailed deer, Wild et al., (2002) reported that an early technique produced many samples without lymphoid follicles and some with >25, while their later technique more consistently produced samples with ≥3 follicles. Wolfe et al., (2002) experimented with various techniques in mule deer and found that when using a mouth gag and a 6-mm biopsy cup and collecting from the rostral rim of the sinus, 155/161 (96%) had ≥1 follicle. Wolfe et al., (2004) collected 201 biopsy samples from mule deer; 10% contained no follicles, 20.1% had 1-8 follicles, and 69.9% had ≥9 follicles. The number of tonsil follicles considered necessary for a negative CWD diagnosis in individual deer was ≥3 in Wild et al.,'s (2002) study and ≥9 by Wolfe et al., (2002). A minimum of 6 follicles is considered necessary for accurate CWD testing of RLNs or PLNs (T. Cornish, Wyoming State Veterinary Laboratory, personal communication).

Wolfe et al., (2002) reported that in tonsil samples with CWD-positive tissue, the percentage of follicles that stained positive varied from 30-100% (average 83%) in post-mortem sampling and from 40-100% in live-animal sampling, while Wild et al., (2002) found that all follicles within a sample stained uniformly positive or uniformly negative in 74 of 75 usable samples. These differences are explained by lower PrPCWD accumulation early in the course of disease and of natural variation in the pathogenesis of CWD (Miller and Williams, 2002). Variation in staining of lymph node follicles and localization of prion accumulation to one pole of the node has also been observed in RLNs of CWD-positive animals (Hibler et al., 2003).

5. Conclusions

Our evaluations indicate that PLN collection is efficient for hunter-harvested and live-animal collections. In addition to reducing time in contrast to RLN collection, supplies and training required were minimal and success of collecting usable samples was high. For live animals, we noted some difficulties with sample collection (e.g., collection of nontarget tissue). Despite those drawbacks, we collected useable PLN tissue samples from our procedures in live deer. There is a need for studies of PrPCWD presence in PLN at various stages of CWD to better understand the utility of this procedure. Other less-invasive techniques, such as the testing of blood and urine, should continue to be explored for live-animal CWD surveillance efforts (Hunter et al., 2002; Schmerr et al., 1999, Schmerr 2002; Shaked et al., 2001).

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6. Sources

Allen, M. J., and G. L. Borkowski. 1999. Veterinary Care. Pages 49–98 in M. A. Suckow, editor. The Laboratory Small Ruminant. CRC Press, New York, New York, USA.

Beringer, J., L. P. Hansen, J. J. Millspaugh, and T. Meyer. 2003. A statewide surveillance effort for detecting chronic wasting disease in wild white-tailed deer in Missouri. Wildlife Society Bulletin 31:873-881.

Conner, M. M., C. W. McCarty, and M. W. Miller. 2000. Detection of bias in harvest-based estimates of chronic wasting disease prevalence in mule deer. Journal of Wildlife Diseases 36:691-699.

Diefenbach, D. R., C. S. Rosenberry, and R. C. Boyd. 2004. Efficacy of detecting chronic wasting disease via sampling hunter-killed white-tailed deer. Wildlife Society Bulletin 32:267-272.

Hadlow, W. J., R. C. Kennedy, and R. E. Race. 1982. Natural infection of Suffolk sheep with scrapie virus. Journal of Infectious Diseases 146:657-664.

Hibler, C. P., K. L. Wilson, T. R. Spraker, M. W. Miller, R. R. Zink, L. L. DeBuse, E. Anderson, D. Schweitzer, J. A. Kennedy, L. A. Baeten, J. F. Smeltzer, M. D. Salman and B. E. Powers. 2003. Field validation and assessment of an enzyme-linked immunosorbent assay for detecting chronic wasting disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginanus*), and Rocky Mountain elk (*Cervus elaphus nelsoni*). Journal of Veterinary Diagnostic Investigation 15:311-319.

Hunter, N., J. Foster, and A. Chong. 2002. Transmission of prion diseases by blood transfusion. Journal of General Virology 83:2897-2905.

Lin, H.-C., and D. G. Pugh. 2002. Anesthetic Management. Pages 405–419 in D. G. Pugh, editor. Sheep and Goat Medicine. W. B. Saunders, Philadelphia, Pennsylvania, USA.

Mackie, R. J., J. G. Kie, D. F. Pac, and K. L. Hamlin. 2003. Mule deer. Pages 889-905 in G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild Mammals of North America: Biology, Management, and Conservation. Second edition,. John Hopkins University Press, Baltimore, Maryland, USA.

Miller, M. W., and E. S. Williams. 2002. Detection of PrPCWD in mule deer by immunohistochemistry of lymphoid tissues. Veterinary Record 151:610-612.

Petersburg, M. L., A. W. Alldredge, and W. J. de Vergie. 2000. Emigration and survival of 2-year-old male elk in northwestern Colorado. Wildlife Society Bulletin 28:708-716.

Saar, L. I., and R. Getty. 1975. Ruminant lymphatic system. Pages 1024-1064 in R. Getty. Sisson and Grossman's The Anatomy of the Domestic Animals. Fifth edition. W. B. Saunders, Philadelphia, Pennsylvania, USA.

Samuel, M. D., D. O. Joly, M. A. Wild, S. D. Wright, D. L. Otis, R. W. Werge, and M. W. Miller. 2003. Surveillance strategies for detection chronic wasting disease in free-ranging deer and elk. United States Geological Survey, National Wildlife Health Center, Madison, Wisconsin, USA.

Schmerr, M. J., A. L. Jenny, M. S. Bulgin, J. M. Miller, A. N. Hamir, R. C. Cutlip, and K. R. Goodwin. 1999. Use of capillary electrophoresis and fluorescent labeled peptides to detect the abnormal prion protein in the blood of animals that are infected with a transmissible spongiform encephalopathy. Journal of Chromatography A 853:207-214.

Schmerr, M. J. 2002. Detection of the abnormal prion protein in blood. Page U115 in Abstracts of papers of the American Chemical Society, April 2002.

Schummer, A. H. Wilkens, B. Vollmerhaus, and K. H. Habermehl. 1981. Lymph nodes and lymph collecting ducts of the ox. Pages 383-404 in R. Nickel, A. Schummer and E. Seiferle, editors. The Anatomy of Domestic Animals. Volume 3. Springer-Verlag, New York, USA.

Shaked, G. M., Y. Shaken, Z. Kariv-Inbal, M. Halimi, I. Avraham, and R. Gabizon 2001. A protease-resistant prion protein isoform is present in urine of animals and humans affected with prion diseases. Journal of Biological Chemistry 276:31479-31482.

Spraker, T. R., R. R. Zink, B. A. Cummings, M. A. Wild, and K. I. O'Rourke. 2002. Comparison of histological lesions and immunohistochemical staining of proteinase-resistant prion protein in a naturally occurring spongiform encephalopathy of free-ranging mule deer (*Odocoileus hemionus*) with those of chronic wasting disease of captive mule deer. Veterinary Pathology 39:110-119.

Wild, M. A., T. R. Spraker, C. J. Sigurdson, K. I. O'Rourke, and M. W. Miller. 2002. Preclinical diagnosis of chronic wasting disease in captive mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginanus*) using tonsillar biopsy. Journal of General Virology 83:2629-2634.

Williams, E. S., and S. Young. 1980. Chronic wasting disease of captive mule deer: A spongiform encephalopathy. Journal of Wildlife Diseases 16:89-98.

Williams, E. S., and S. Young. 1982. Spongiform encephalopathy of Rocky Mountain elk. Journal of Wildlife Diseases 18:465-471.

Williams, E. S., and S. Young. 1992. Spongiform encephalopathies of Cervidae. Scientific and Technical Review. Office of International Epizootics 11:551-567.

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

Wolfe, L. L., M. M. Conner, T. H. Baker, V. J. Dreitz, K. P. Burnham, E. S. Williams, N. T. Hobbs, and M. W. Miller. 2002. Evaluation of antemortem sampling to estimate chronic wasting disease prevalence in free-ranging mule deer. Journal of Wildlife Management 66:564-573.

Wolfe, L. L., M. W. Miller, and E. S. Williams. 2004. Feasibility of "test-and-cull" for managing chronic wasting disease in urban mule deer. Wildlife Society Bulletin 32:500-505.