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Stopping the Spread

MANAGERS ADOPT NEW STRATEGIES TO COMBAT INVASIVE SPECIES



High-caliber conservation
Diagnostic labs key to fighting CWD
Two decades of success for State
and Tribal Wildlife Grants



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Getting Results in the Fight Against CWD

DIAGNOSTIC LABS ARE A KEY PARTNER IN MANAGING CHRONIC WASTING DISEASE

By Krysten Schuler, Rachel Abbott, Jonathan Mawdsley and Kaitlyn McGarvey

Every fall, Ryan Rockefeller makes the rounds at meat processors in his area to check white-tailed deer carcasses, collecting deer heads and pulling samples from them to test for indications of chronic wasting disease.

Donning latex gloves and a surgical gown, Rockefeller uses a scalpel and forceps to extract the lymph nodes from just behind the lower jaw and drops them into a plastic vial. He estimates the age from the eruption and wear of the teeth and marks it on the data card. The following day, Rockefeller, a big game biologist for the Department of Environmental Conservation in western New York state, batches the cards and samples and delivers them to Cornell University's Animal Health Diagnostic Center.

This process is repeated thousands of times across the country by biologists just like him, who have to wait days or even months to find out from diagnostic laboratories if the results are "CWD not detected" or "CWD positive." A positive CWD result

can be devastating. For a hunter, it means having to decide whether or not to follow the Centers for Disease Control's advice and not eat the venison in the freezer. For a state, the repercussions are even greater. Officials will need to decide what actions to take to determine the extent of the infection and manage the disease in its deer population.

Both rely on the expertise of laboratories approved to test for CWD.

Limited labs

In 2017 and 2019, the Association of Fish and Wildlife Agencies surveyed each state agency to identify needs and opportunities for CWD management. The surveys showed a rapidly growing demand for CWD testing across the country as state and federal agencies seek more information about the spread of the disease in wild cervid herds, and hunters want to know the disease status of their harvests.

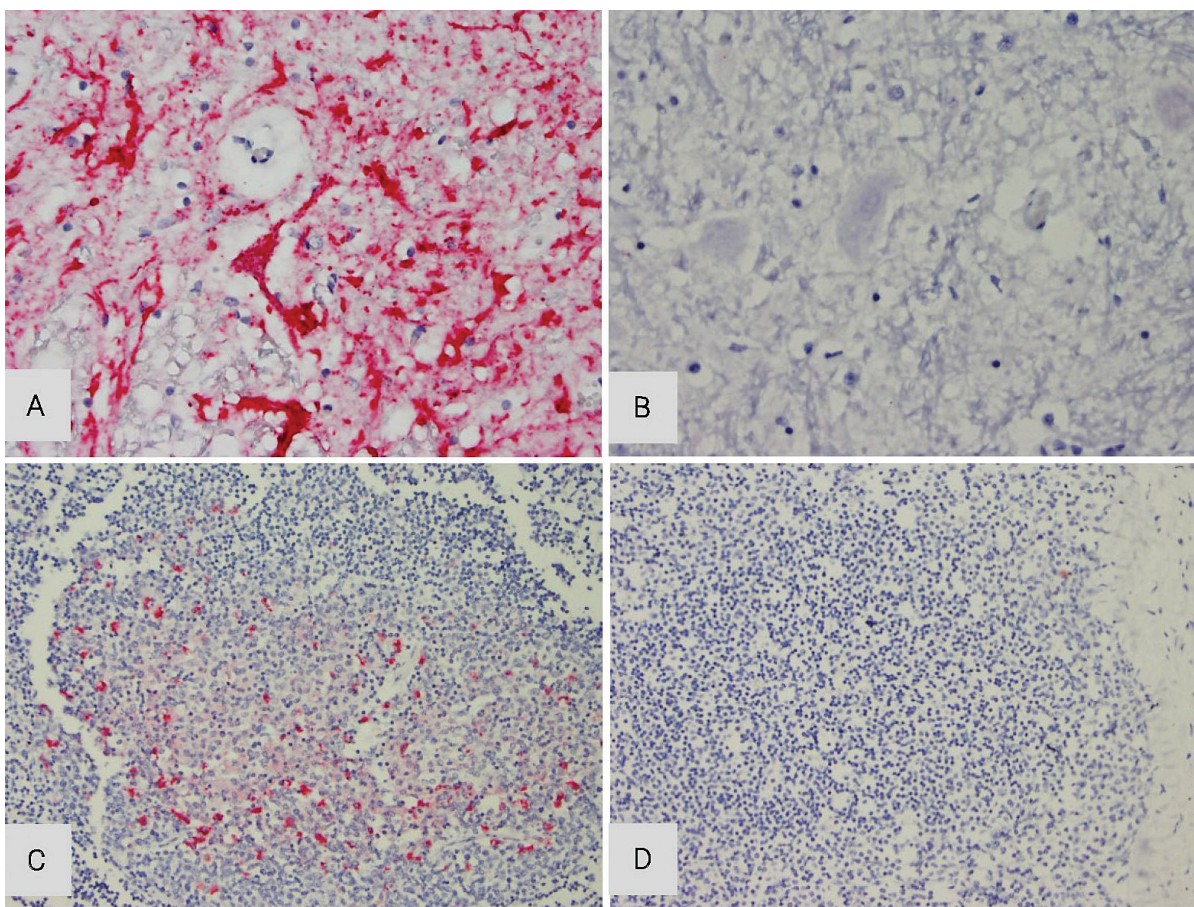
Anecdotal accounts suggested limitations on the availability of testing and delays returning test results. A survey of accredited diagnostic labs would provide useful information on the current status of CWD testing and future capacity.

Diagnostic labs are often associated with a state's veterinary school or agricultural agency. Some state wildlife agencies have their own facilities. There are national and regional wildlife health labs, and the USDA-National Veterinary Services Lab confirms new detections of CWD. In addition to CWD, these labs can test for hundreds of other health parameters, pathogens and parasites in a variety of species. Samples are typically submitted by veterinarians or



◀ A) Krysten Schuler collects medial retropharyngeal lymph nodes and obex from the head of a white-tailed deer harvested in New York. B) Lymph nodes must be trimmed to fit in a cassette that will start the IHC testing process. C) IHC-stained glass slides with fixed tissues wait to be examined under a microscope by a trained pathologist to determine if the deer was infected with chronic wasting disease.

Courtesy Benjamin Jakobek/Cornell University



◀ A microscopic view of tissues stained by IHC. A) CWD-positive obex by IHC. The red staining indicates the presence of CWD prions. B) CWD was not detected in this brain section by IHC. C) CWD-positive lymphoid tissue by IHC. Red staining is observable in the follicles of the lymph nodes. D) CWD was not detected in this lymph node sample by IHC.

Courtesy T. Southard/Cornell University

agency personnel rather than directly by domestic animal owners or hunters.

Across the country, there are [28 of these labs](#), all certified by the [National Animal Health Laboratory Network](#), which is overseen by the U.S. Department of Agriculture. These labs follow strict protocols to ensure the reliability of their results. Ideally, a test should be both sensitive and specific. A highly sensitive test is more likely to detect an infection if it exists, so fewer cases of disease will be missed. A highly specific test is unlikely to indicate an infection exists when in reality it does not, so hunters and states won't be alarmed unnecessarily.

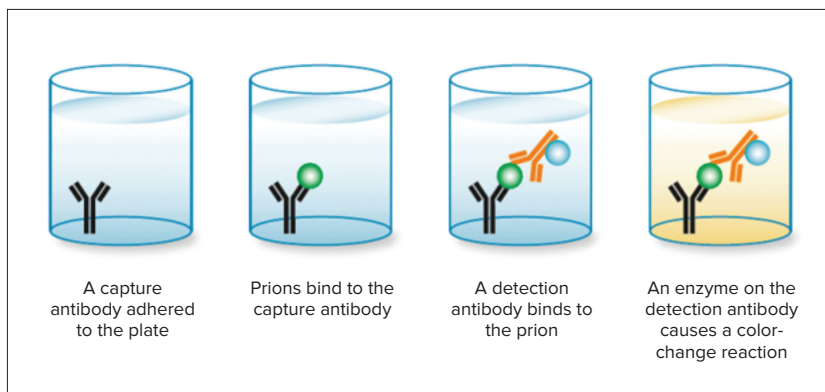
Two tests

Two types of tests are currently approved for post-mortem diagnosis of CWD: immunohistochemistry (IHC) and enzyme-linked immunosorbent assay (ELISA). Both tests are validated for specific tissues: obex — or brainstem — tissue or the retropharyngeal lymph nodes, which are found close to the intersection of the head and neck.

IHC is often used as a confirmatory test and is considered the “gold standard” because of its high specificity, meaning few false positives. It uses formalin-fixed tissues on slides. A series of antibodies bind to abnormal prions if they are present, and a colored stain attaches to the antibodies. A trained pathologist looks for the colored tissues, indicating a positive result, under a microscope.

What is CWD?

Chronic Wasting Disease (CWD) is a transmissible spongiform encephalopathy causing neurologic disease in mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), elk (*Cervus canadensis*) and moose (*Alces alces*). It is caused by a prion, an abnormal form of a cellular protein that is most commonly found in the central nervous system and in lymphoid tissue. Prions “infect” host animals by promoting misfolding of the host's normal proteins.



Credit: Jennifer Peasley/Cornell Wildlife Health Lab

▲ **ELISA tests use antibodies that generate a color change at the end of the process to indicate that prion proteins are present.**

ELISA is done on fresh tissue and also uses antibodies and a color change to indicate the presence of abnormal prions. Since tissues are put in tubes, not affixed to slides, ELISA allows labs to process larger numbers of samples at a time. A machine reads the color-change reactions automatically. Depending on the state and situation, samples that test positive by the ELISA may be subsequently confirmed by IHC.

A question of capacity

To better understand CWD testing capacity in the U.S., we contacted all the USDA-approved labs to request information on testing for sampling year 2018-2019 and areas for improvement. We received responses from 22 of the 28 labs. Six labs run only IHC, eight have ELISA and eight have both tests available.

One of the most common issues labs reported was that one of the pieces of IHC equipment used for staining slides was no longer supported by the manufacturer, leaving many labs unable to conduct the test. There were also delays due to expensive and backordered reagents. For ELISA, the major problems were having enough staff to prepare the samples, availability of test kits and outdated operating software for the machine that reads the results.

CATEGORY	IHC	ELISA
Wild cervids	25,350	159,860
Captive cervids	30,070	3,340

▲ **Numbers of wild and captive cervids reported as tested by IHC and ELISA in the 2018-2019 sampling season for 22 of the 28 USDA-approved CWD testing diagnostic laboratories. IHC is the approved CWD test method for routine herd surveillance of postmortem samples and epidemiological investigations of captive cervids. ELISA is more commonly used on postmortem samples collected from hunter-harvested wild cervids.**

Although the public often asks why all deer, elk and moose aren't tested for CWD, states do not need to do a complete census of all animals to understand the prevalence of the disease. Surveillance programs can be designed to sample enough animals in order to provide reasonable assurance that if CWD is present in the population then testing will be able to detect it. Selective testing of high-risk animals can be incorporated into the surveillance system to reduce the number of animals that need to be tested.

Despite calls from the public and agencies for more testing, feedback from the labs indicates that most wanted their testing capacity to remain the same. A mismatch in public need versus available testing capacity may be challenging for state wildlife agencies, particularly when the agency is funding the testing. We also heard that only one company provides equipment for the USDA-approved CWD ELISA testing, and more diversity is needed in that area.

Other tests conducted in research labs — protein misfolding cyclical amplification (PMCA) and real-time quaking-induced conversion (RT-QuIC) — would be of interest to diagnostic labs if the tests were approved by NAHLN, because they may be more sensitive for detecting infections, particularly early in the course of the disease. The RT-QuIC is currently being evaluated as a diagnostic test by the USDA.

Turnaround

Different tests and tissues are used for CWD surveillance depending on species, whether the animal is wild or captive, and time of year. At the Animal Health Diagnostic Center, we only conduct ELISA testing during the hunting season for free-ranging white-tailed deer and run IHC on suspect wild deer, wild moose and captive cervids throughout the year. The reason is that we are able to process large batches of samples more efficiently with ELISA, but this test is not as cost-effective for small numbers of samples. IHC samples are processed individually, which typically takes longer to deliver results. The costs of the tests are similar.

Fifteen of the labs that responded to the survey will test animals submitted directly by hunters. In most cases, the hunters have to pay the fee, which averages about \$48 but can be as high as \$80. The remaining labs only test animals submitted by



Credit: Nick Hollingshead/Cornell Wildlife Health Lab

state agencies. Some agencies will assist with or provide training on sample collection by hunters and facilitate submission to the lab. These services vary by state and disease management units so it is important for hunters to check with the state agency or lab prior to the start of hunting season.

Diagnostic testing often takes one to two weeks to complete, but turnaround times may be longer during hunting season, when most samples are collected. States like Tennessee and Montana, which have newly-detected CWD, are assisting with establishing in-state testing laboratories to speed delivery of results. Ideally, state wildlife agencies and diagnostic labs can work together to find solutions to improve results reporting and meet the public's needs. Many agencies have already stepped up to support their state's veterinary diagnostic lab by purchasing equipment, streamlining sample submission and ensuring testing processes are more transparent and accessible.

Five days after Rockefeller submitted his samples, Cornell's Animal Health Diagnostic Center sent the results to the DEC Wildlife Health Unit with all the testing conducted that week. It was good news. Rockefeller had reached the sampling goals for his

region, and the report he received told him all of the deer were “CWD not detected.”

Across the country, other biologists will be conducting this same process and receiving their results. In areas where CWD has existed previously, a positive result may not be surprising. For others, the first CWD detection in their area represents a point of no return. It marks the arrival of a disease for which there is no cure, and it comes with far-reaching impacts on many aspects of wildlife management. ■



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