Fight Against CWD Advances As New Technologies Come On-Line

by COLLEEN SCHREIBER | Apr 28, 2022

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KINGSVILLE — Chronic Wasting Disease (CWD), an infectious, degenerative disease in cervids, is a growing concern throughout the nation.

According to the National Wildlife Health Center, 29 states have CWD in free-ranging cervid populations and 18 states with captive cervid populations.

While there may be some cervids with resistant genes that slow the progression of the disease, the disease is always fatal. According to Dr. Peter Larsen, winning the war against CWD will only happen with next-generation diagnostics, active surveillance and therapeutics.

That was the message he shared to those attending the recent Deer Associates annual gathering hosted by the Caesar Kleberg Wildlife Research Institute. Larsen is the co-director of the Minnesota Center for Prion Research and Outreach (MNPRO), a multidisciplinary think tank, which he helped found in 2019. Over the last three years, MNPRO has developed new technologies that are expected to provide options in the fight against CWD.

Larsen told the group that MNPRO came about, in large part, because Minnesota legislators kept asking questions about the CWD diagnostic tools. Specifically, they wanted to know why the diagnostic options were so limited and why prions couldn't be detected in the environment – questions that the cervid industry had long been asking as well. Larsen didn't have good answers for the legislators, but he made a personal commitment to get them. He recognized that the only way researchers, managers and deer enthusiasts would ever be able to finally get ahead of CWD and begin to "start fighting the war effectively" was to have a focused research effort aimed at developing next generation technologies. It was with that thought in mind that MNPRO was created.

An initial $2 million grant from the state of Minnesota was used to build a state-of-the-art advanced prion research lab completed in a short five months. Today, there are some 40 research affiliates across multiple universities, state agencies, tribal nations working on developing next-gen cutting edge technologies to fight prion protein misfolding diseases like CWD as well as Creutzfeldt-Jakob disease, Alzheimer's and Parkinson's.

Before getting into some of the research, Larsen offered a brief overview of just what a prion is. All mammals have prion proteins, he told the group. Normal protein prions perform routine physiological activities. For example, they help regulate copper, zinc and iron metabolism.

If a normal prion could be compared to a slinky toy, then a CWD prion would be a "misfolded messed up slinky," said Larsen. "It has completely lost its structure. It no longer is a three-dimensional structure that has a function."

When an abnormal prion comes into contact with a normal prion, it causes the normal protein to change its shape. Specifically, it begins to misfold resulting in a chain reaction of misfolds over time.

Specific to CWD, it takes about two years for this domino effect to make its way up into the brain of the deer and into different tissues of the body. In the late stages of the disease, nerves are destroyed resulting in the neurological effects.

Larsen also confirmed what has been proven, that these prions are spread animal to animal, as well as from animal to environment to animal. Additionally, these infected prions are resistant to degradation and can remain infectious in the environment for several years. Research in Colorado has proven that in a captive mule deer facility remained contaminated after five years of vacancy.

Turning then to the current diagnostic tools, Larsen acknowledged that the traditional tests, ELISA and IHC, while they work at detecting infection, also have lots of limitations. For example, this year there was a shortage of test kits available. Currently, it happens to be the ELISA kit that's short. Last fall, there was a major shortage of the IHC kits. He said that Minnesota hunters often had to wait several weeks before getting CWD test results.

Larsen said that these traditional tests are expensive and large labs are needed to perform the diagnostic work. Additionally, early-stage infections may be missed with these diagnostic tools.

There is a second-generation test now available, he said. It's known as Real-Time Quaking Induced Conversion (RT-QuIC). Originally developed for CJD in humans in 2007, it is currently undergoing USDA valuation for CWD testing as well.

The RT-QuIC test monitors protein misfolding in real-time. It is also a "highly sensitive" test with reproducible results.

He shared results from a study done with the Minnesota Department of Natural Resources. DNR tested 500 white-tailed deer for CWD using the ELISA and IHC tests. They found 12 positives. The MNPRO lab then took those samples and ran the RT-QuIC test. It was a blind test meaning they did not know in advance which ones were positive. The RT-QuIC test found the 12 positives plus three additional which were thought to perhaps be indicative of early-stage infection.

He stressed that there was no evidence of false positives with the test. Also, this study confirmed that the test sensitivity and specificity is excellent.

"This test is so sensitive that you could take one tablespoon of these misfolded prions mix it into an Olympic sized swimming pool and still find the positives," Larsen insisted.

He said that USDA expects to complete the validation of the test for CWD within the year and already more and more labs are getting the RT-QuIC test in anticipation of that approval.

All of this work was done in 2019. By 2020, MNPRO was already looking to developing a field deployable second-generation test. A breakthrough came in October 2020, when a graduate student, Peter Christenson, discovered that prions bind to gold nanoparticles. It is gold nanoparticles that are used in pregnancy tests, even the rapid COVID test. It is the gold nanoparticles that cause the sample to turn a different color if the test is positive. No one had ever tried it for CWD. The graduate student found a clear color difference, seen with the naked eye, with positive CWD samples.

"We've run hundreds and hundreds of tests with the same reaction," said Larsen. "It is 100 percent in agreement with RT-QuIC results and the IHC and ELISA results on those same tissues."

The new test, known as MN-QuIC, is now undergoing formal validation as well. The hope is to submit it to USDA for validation in mid to late 2022.

Larsen told the group that not only is the test field deployable, but it uses much less expensive equipment. In fact, the RT-QuIC equipment is $28,000 versus $3000 for the MN-QuIC test. The RT-QuIC test costs about $15 per sample; MN-QuIC is $3.

With that in their pocket, MNPRO is already working on third generation CWD technologies. One is a handheld device the size of a cell phone that can detect CWD in the field in four hours. They're in the process of filing a provisional patent on this technology as well.

"We think these field deployable tools are feasible and on the horizon," he told the group.

Still another line of research is focusing on using these technologies to help manage CWD in the environment.

"We know that CWD prions are found on many different surfaces out in the environment," he said. "We're looking to see if the CWD prions bind to any of these surfaces in different ways."

Already they've learned that these prions readily bind to steel surfaces. Thus, in collaboration with the University of Creighton, a swabbing technique was developed for testing of steel surfaces. The idea then was to have some steel plates, what Larsen referred to as CWD sentinels, to be placed strategically in feeders or troughs or perhaps near water.

They tested this in a pen situation with known CWD positive deer. After a week of licking on these "sentinels," they were swabbed and sent back to the lab to be tested using the RT-QuIC method.

In Pen 1, 13 of the 19 deer were known to be CWD positive based on the IHC test. In Pen 2 only one out of 12 was positive for CWD with the IHC test.

The CWD sentinel results showed this as well but in a different way. In Pen 1, where there were 13 of 19 known CWD positives, four different sentinels came back "screaming hot" based on the RT-QuIC test.

In Pen 2, it was less so, but even with just the one known positive, it was clear that animal was depositing infectious prions on the steel plates at a detectable level using the swab and the RT-QuIC test.

"This sentinel technology opens the door for real-time herd-level surveillance and culling," said Larsen.

The idea would be to deploy these sentinels at feeders and swab and test every few weeks. If a positive swab is detected, then that particular feeder could be monitored more frequently. If more positive swabs come forth, then that would perhaps indicate culling needed to be done for the deer in that area. He said that game camera traps could possibly be used to identify CWD positive individuals. He insisted that rapid portable diagnostic technology sentinels are a very feasible tool.

Larsen also briefly discussed the role of therapeutics in fighting CWD. He reminded that the biological function of normal prions is to help regulate copper, zinc and other metal ions. That suggests that nutrition is a very important component of the CWD puzzle. When CWD was first described, it was considered a copper-related disease in that it was thought to have originated from cervids being in environments with low levels of copper.

"It's been in the scientific literature for a while that there may be a diet component that is really important for disease management of CWD," he told the group.

More recent literature shows that prion proteins bind well to zinc and copper. Another body of scrapie literature indicates that there are certain kinds of copper that can actually bind to and reduce the progression of the daisy chain reaction in the prion. It's been done in a mouse model infected with scrapie, he told the group.

Similarly, this same kind of analysis has been done in a test tube model for CWD and shows promise with similar results to the scrapie work. MNPRO is interested in taking this research to the field.

They've teamed up with Lyssy & Eckel Feeds who produces a feed with chelated copper and zinc feed from the company known as Zinpro. Together they are investigating whether there is a potential inhibitory role of organic copper, feeding Zinpro minerals, in the misfolding and propagation of the CWD prion.

In theory, or the hope is, that feeding this copper and zinc-based feed could slow the progression enough so that there are not super spreaders, so to speak, on the landscape. Then the idea would be to find another smaller molecule that could be used to knock out the misfolded prions completely.

"Another of our lines of research is focused on finding the small molecules that can actually bind to and neutralize this misfolded slinky," Larsen told the group.

Wrapping up, he reiterated that to win the war the enemy must be attacked from multiple fronts with a proactive next generation multi-pronged approach.

"We think the faster portable diagnostics are really essential, because we can have early detection sentinels at the ranch level with targeted culling.

"Then we need science-based nutrition. This is an important part of the CWD puzzle that's been under studied. We need to be better at that," he concluded.