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Wildlife Futures Program
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Senate Agriculture and Rural Affairs and Senate Game and Fisheries Committees
on Chronic Wasting Disease

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Committee Chairs Laughlin, Vogel, Brewster and Schwank, and members of the committees, thank you for the invitation to appear before you today to discuss Chronic Wasting Disease (CWD). As you know, CWD has emerged as a major concern in Pennsylvania since it was first identified here in 2012.

As Pennsylvania's only veterinary school and a global leader in veterinary instruction, clinical services and diagnostic testing, as well as interdisciplinary research, the Pennsylvania Game Commission (PGC) partnered with the University of Pennsylvania School of Veterinary Medicine (Penn Vet) nearly three years ago to use our expertise as a resource to inform the agency's wildlife management decisions and public policies. Our collective goal is to offer a science-based, wildlife health program that strengthens the resilience of Pennsylvania's nearly 480 species of birds and mammals, while at the same time integrating comprehensive disease surveillance and mitigation into a single, unified effort that addresses at-risk wildlife populations.

Although there are numerous diseases impacting the health of Pennsylvania's wildlife populations every day, the Wildlife Futures Program's initial emphasis has been on CWD. The disease—which is caused by a prion, or an infectious protein particle—is a fatal disease in cervids. Through our joint effort, we have greatly expanded the state's diagnostic capacities, facilitated improved field operations, incorporated best-practice modeling, and conducted critical research to improve the tools available to identify and combat CWD. Our testimony today outlines this work.

Improving Diagnostic Capabilities and Validating New Testing Methods

One of the Wildlife Futures Program's first goals was to expand the CWD diagnostic capabilities for Pennsylvania in conjunction with the Pennsylvania Animal Diagnostic Laboratory System (PADLS). With the ever-increasing prevalence of CWD in Pennsylvania, testing demands are projected to increase annually. Additionally, while human susceptibility to CWD is unknown and testing is not considered a valid measure of food safety, it is important to get test results back to hunters quickly so they may make informed decisions about consuming their harvested meat.

After the first three years, the Wildlife Futures Program has expanded Pennsylvania's capacity greatly with the addition of CWD enzyme-linked immunoassay (ELISA) testing in 2019 and updated immunohistochemistry (IHC) diagnostic equipment in 2020. The program currently tests 10,000-15,000 samples per year, with the capacity to test up to 25,000 samples as may be needed. For context, in Fiscal Year 2020-21, the Wildlife Futures Program, working through Penn Vet's state-supported PADLS laboratory at New Bolton Center, tested 12,359 samples as compared 4,483 samples tested in FY 2019-20.

We also have reduced the average turnaround time of these tests. Prior to Wildlife Futures' launch, it was not uncommon for tests to take weeks—or even months—to be reported back to hunters. At its launch in August 2019, we committed to reducing that turnaround time to 10-21 days. We are happy to report that we exceeded that commitment, often reporting results in as little as six days. Recently, however, like many other industries, CWD testing has been challenged by supply chain issues, specifically for reagents used in the testing process. Currently, there is only one CWD ELISA assay approved by the U.S. Department of Agriculture (USDA) for testing. The Wildlife Futures Program has worked to validate wild white-tailed deer testing kits from an additional commercial supplier. With the validation of this method, we are prepared to maintain testing levels even in the event of future supply chain disruptions.

Early detection is a critical CWD management tactic as once the disease is in the environment, prions can last for over a decade, become amplified in the soil, and can be taken up by plants. Further complicating the matter are the realities that clinical signs of CWD can take up to two years to develop and available diagnostic tools do not detect CWD until 6-12 months post-infection—and only on a postmortem basis as there are no approved antemortem tests.

The gold standard for CWD diagnostics is IHC testing, which is time consuming, expensive, and requires a board-certified veterinary pathologist. Improved diagnostic techniques that can detect infections earlier, can be conducted more rapidly, and are relatively inexpensive will improve the capacity for CWD surveillance.

Real-Time Quaking Induced Conversion (RT-QuIC) can detect CWD prions at lower concentrations and thus earlier in the disease progression. This assay has been shown to detect infected animals four months prior to other methods and is effective at up to 10^{-14} fold dilutions. The ability to detect infected animals earlier in the disease progression, as well as the potential to pool samples, is beneficial to CWD surveillance as it permits earlier detection and the ability to test more samples for less expense. Thus, RT-QuIC has the potential to aide surveillance efforts and allow for more rapid intervention to keep CWD from spreading to new areas.

Despite a substantial amount of research to date on the use of RT-QuIC, several difficulties have limited application in diagnostic settings. An effort to find optimal protocols and laboratory conditions for RT-QuIC by certified diagnostic laboratories is needed to move this promising technique from research into applied use. To accomplish this goal, the Wildlife Futures Program laboratory has developed RT-QuIC capacity and validated the assay against current diagnostic standards. The major outcomes of this work are:

1. We have shown RT-QuIC is a reliable diagnostic approach using retropharyngeal lymph nodes;

2. We have confirmed the availability and accuracy of the commercially available substrate; and

3. We have optimized the reaction, sample concentrations, and run time for use in a diagnostic setting.

This work has the potential to allow for greater testing capacity for white-tailed deer in Pennsylvania and represents a significant step forward that demonstrates the value of this relatively nascent partnership between Penn Vet and PGC.

Adding Wildlife Health Support in the Field

As part of the Wildlife Futures Program, Penn Vet hired six Wildlife Health Technicians (WHTs) who are located throughout the Commonwealth—one in each of the PGC's six regions. Each WHT is a Certified CWD Technician (CCT) and each participates in CWD surveillance.

Each hunting season, our WHTs assist with collecting samples from a number of sources, including hunter-harvested deer at processors and from head-bin collection sites. Year round they assist with collections from escaped captive deer upon capture and suspected clinical cases; or (upon the request of USDA and/or PGC) following targeted removal exercises on private property or Disease Management Areas (DMAs). Depending on the circumstance in question, WHTs extract lymph nodes, conduct field necropsies, package samples, enter data into the appropriate database, ship or deliver samples to the appropriate lab, and dispose of the head remains.

In particular, the WHT in PGC's northcentral region assists in CWD surveillance of elk populations by extracting lymph node and obex samples at check stations and from all non-hunting elk mortality cases with which she is involved, and by shipping samples extracted by other PGC trained personnel. She enters this data into Flowfinity (for hunter-harvested deer) or WHIN (for non-hunter-harvested deer), and ships or delivers samples to Wildlife Futures.

With their extensive training and certifications, these efforts by WHTs are critical to obtaining samples in a timely manner and of sufficient quality for diagnostic analyses.

Improved CWD Modeling and Surveillance

CWD management is likely to be most effective if enacted when the disease has not spread widely, and the prevalence is low. Early detection is key but detecting a few infected deer among millions in Pennsylvania is a major barrier to effective surveillance.

To generate the optimal surveillance strategy for Pennsylvania (i.e., one that maximizes the chances of detecting CWD while minimizing cost), the PGC and Wildlife Futures partnered with Cornell's Surveillance Optimization Project for Chronic Wasting Disease (SOP4CWD), which incorporates the best available data with industry-leading mathematical models to determine the optimal surveillance strategies that will minimize costs and maximize the likelihood of detection.

SOP4CWD's analyses identify which geographic locations are at highest risk for CWD, and then determines which can be tested given available financial resources. The resulting output gives managers the best opportunity to find CWD quickly in new areas and to detect an increase in prevalence within areas known to have CWD-positive cases.

An additional challenge is determining how many samples are needed to detect CWD at $\leq$1% prevalence. Currently, there are two approaches: the Walsh and Miller method and Belsare method. The Walsh and Miller sampling quotas provided the best model-informed surveillance

targets at the time and are the targets commonly used by state game agencies. However, the approach assumes an even distribution of infected animals, when CWD is normally clustered geographically. A more recent approach by Belsare demonstrated that clustering of CWD-positive animals results in an overestimation of the confidence of detecting CWD using the Walsh and Miller framework.

In determining the optimal CWD surveillance for Pennsylvania, we ran analyses with the Belsare and Walsh and Miller frameworks. In doing so, our model recommends not sampling in a county if the goal of detection cannot be met financially. The collaboration with SOP4CWD has provided informative results that highlight areas where increased CWD surveillance is recommended and demonstrate how PGC is incorporating best practices into modeling as part of its CWD surveillance plan.

CWD surveillance is complex, however, and the mathematically optimal surveillance does not equate with the most realistic or even best surveillance plan for Pennsylvania. Several factors contribute to CWD surveillance in Pennsylvania, including logistical challenges and costs associated with expanded surveillance, the need to serve all stakeholders to the fullest extent possible, and risk factors not easily captured within the current modeling framework. Most importantly, currently the testing budget is not limited so we are focusing efforts on risk modeling instead of optimization. Future efforts will focus upon improving risk data to inform these models and continuing close collaboration with the Cornell team. Continued collaboration will allow PGC to incorporate new data annually and work with the most up-to-date risk maps. This will best position Pennsylvania to identify CWD in new areas and enable swift management interventions.

Researching New Ways to Detect CWD

Novel Biomarkers in Fecal Microbiome

Chronic Wasting Disease (CWD) can propagate through oral ingestion of animal tissues and by-products containing infectious prions, thus making a deer's gastrointestinal tract the primary route of infection. Ingesting prions is hypothesized to affect gut microbiota, a multi-species population of symbiotic and pathogenic microorganisms that reside in the intestines of every animal. Penn Vet's researchers have analyzed CWD-dependent changes in gut microbiota using feces, which contain a representative sample of bacteria from each individual animal and can be collected easily without capturing deer.

In the absence of a reliable antemortem test, identifying feces-based CWD diagnostic markers could provide a perfect tool for disease surveillance and control. Studies analyzing fecal samples from deer with CWD are emerging in the field, but currently no robust biomarkers that can inform disease surveillance, diagnostics, and effects on normal animal physiology have been identified. Our goal is to identify such biomarkers.

Our researchers have used a set of fecal samples obtained from deer collected in different regions of the country, including equal numbers of CWD-positive animals of different genders and healthy controls, to identify CWD-dependent changes in their microbial composition. Using high throughput sequencing, we identified 64 bacterial taxa that show differential abundance between

CWD and control. A subset of these taxa are highly abundant, and thus represent promising targets for disease surveillance and diagnostics.

With future research, we plan to investigate the use of identified fecal microbiomic signatures of CWD as novel tools for disease surveillance in the wild. Identification of these signatures could be achieved by a simple and sensitive PCR test on fecal samples' DNA preparations. Our next step is to work on optimizing this test for ease and robustness, followed by applying this test to a wider range of deer fecal samples. In the end, the aim is to develop a robust and rapid assay for diagnostics and surveillance of CWD.

Proteins and Metabolites in Deer Muscle

In a similar vein, Penn Vet is now engaged in research to create a CWD field diagnostic test by studying deer muscle. Our research is analyzing deer muscle samples collected in the wild for composition of proteins and major metabolites. This work has identified several potential markers related to changes in the mitochondria and energy metabolism, as well post-translational modifications. Researchers are currently validating these targets for their diagnostic potential. Longer term, we aim to select several robust targets that can be used for disease surveillance in the wild and in meat processing plants to diagnose CWD by a simple hand-held assay kit.

Canine CWD Scent Detection

The Wildlife Futures Program also is leveraging the research and training expertise of the Penn Vet Working Dog Center (PVWDC) to assess whether canines are capable of detecting CWD. The goal of this research, which has been supported with a grant from the Pennsylvania Department of Agriculture, is to train dogs to enhance and assist PGC personnel in the field with their response and management functions.

In August 2020, the PVWDC began researching whether trained scent-detection canines could be used as an antemortem test for CWD based on the volatile organic compounds present in feces from deer with the disease. This initial work provided a solid proof-of-concept, allowing us to move forward with subsequent training. Ultimately, three dogs were selected from a larger pool of candidates for this more intensive training—each with a dedicated handler—that includes odor work, fitness and endurance, direction and control, agility, and obedience.

Upon the completion of training, these three canine teams will work in conjunction with the PGC CWD team in containment zones, Disease Management Areas subject to potential border adjustments or elimination, and areas where there are clinical suspect cases.

Conclusion

Chairpersons Laughlin, Vogel, Brewster and Schwank, and members of the committees, thank you for the opportunity to testify before you. We recognize CWD is a very real animal health threat and a concern for Pennsylvania's hunters. Penn Vet has been a partner of the Commonwealth since 1889. Traditionally, this partnership has focused on agriculture, the health of food animals and the safety and integrity of our food supply. Our recent joint effort with the Game Commission has greatly expanded our role into the wildlife management space, which we believe is critically important to protect against diseases beyond just Chronic Wasting Disease.

Specific to CWD, though, as our testimony today demonstrates, we have made considerable progress on a number of fronts and have made promising advancements in our work to protect against this disease through improved testing and surveillance. We remain available to serve as a resource to you and we look forward to continuing this important work.