UNIVERSITY OF MINNESOTA

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Center for Infectious Disease Research and Policy

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Representative Jamie Becker-Finn
559 State Office Building
St. Paul, MN 55155

Senator Kelly L. Morrison
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Dear Representative Becker-Finn and Senator Morrison:

We believe it is important for Minnesota legislators to be aware of the cutting-edge science regarding the state-of-the-art information on chronic wasting disease (CWD) diagnostics and misconceptions surrounding the breeding of prion-resistant cervids (members of the deer family). Both of these issues are being discussed in association with HF1202/SF1526; however, important scientific information has largely been absent in these discussions.

First, we address the current state-of-the-art science regarding CWD prion testing and specifically respond to a letter sent from Dr. Mark Lyons with USDA APHIS Veterinary Services to the Minnesota Board of Animal Health expressing opposition to proposed legislation that would leverage antemortem use (that is, on living animals) of real-time quaking-induced conversion (RT-QuIC) testing to monitor the state's farmed white-tailed deer for CWD. Although we acknowledge the role that USDA and APHIS Veterinary Services have in the security and preservation of the nation's agricultural commodities, the concerns raised in Dr. Lyons' letter are largely misguided, lack considerable context, and selectively omit a great deal of recent CWD prion testing research.

We believe that the state of Minnesota has the legal right to establish precautionary live-animal CWD surveillance measures that (1) stabilize the cervid farming industry and (2) trigger traditional regulatory testing as part of the national cervid health program. While we are sure that we all agree that control of CWD is paramount to our collective efforts, it is imperative we address this from a scientific perspective and consider the ever-growing totality of evidence and data that exist. Please find attached a current review that addresses RT-QuIC. The conclusions of this review reflect the current knowledge of the leading experts in next-generation prion diagnostics and CWD epidemiology.

Second, we address the misconception that cervid farmers are currently breeding animals with complete genetic resistance to CWD infection. Our attached document provides a comprehensive assessment of genetic susceptibility to CWD. Regardless of genetic background, CWD is 100% fatal for white-tailed deer. No deer are immune to CWD, and breeding efforts must consider unique aspects of CWD biology.

Sincerely,

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Diagnostic Accuracy of RT-QuIC and Utility for Live-Animal CWD Testing

The costs associated with chronic wasting disease (CWD) detection, management, and mitigation are significant for both state[1] and federal[2] agencies. As the disease continues to spread, these costs are expected to rise accordingly. Aside from the tangible, fiduciary losses associated with CWD spread, the cultural and social impacts of CWD should be held in equal regard. Even more fundamentally, venison, elk, and moose meat are critical sources of nutrition in rural, indigenous, and other food-insecure communities[3]. **In light of recent events in Minnesota and across other states, it is abundantly clear that traditional CWD diagnostic tests are failing to proactively prevent human-mediated spread of the disease.** Given the potentially catastrophic impact of CWD to state and national economies, alongside growing concerns of CWD spread to humans, state and federal agencies must act with urgency to establish new diagnostic testing procedures that (1) are based on solid peer-reviewed science and (2) proactively mitigate human-mediated spread of the disease in animals.

The real-time quaking-induced conversion (RT-QuIC) assay is a robust, repeatable, and sensitive method of testing for the presence of misfolded prions. RT-QuIC was originally developed in 2010 for diagnosing prion diseases in both humans and animals.[4,5] Over the past decade, RT-QuIC has routinely been shown to be a powerful assay for detecting CWD across a variety of tissue types. Several peer-reviewed studies have directly compared RT-QuIC performance to current regulatory antibody-based tests (e.g., ELISA, IHC).[6–10] These studies show that the RT-QuIC assay consistently provides comparable results to the antibody-based tests, with low rates of false-positives and false-negatives. Moreover, RT-QuIC demonstrates magnitudes greater sensitivity than current regulatory CWD tests.[11] Because of this increased sensitivity, RT-QuIC can detect CWD in deer months before it can be detected by traditional tests.[12]

A growing body of evidence shows that RT-QuIC is useful for screening both live and dead cervids (members of the deer family) and cervid by-products for CWD prions.[7,8,13–16] With respect to live-animal testing, a series of published studies have documented that RT-QuIC effectively detects CWD prions within a variety of tissues, including skin, tonsil, and eyelid biopsies, with test sensitivity (i.e., the probability that the test will detect a positive animal) ranging from 72% to 96% and test specificity (i.e., the probability that an uninfected animal will test negative) ranging from 91% to 100%.[6–10,13,14,17]

HF1202/SF1526 references the use of RT-QuIC testing of ear skin biopsies as an initial live-animal screening tool with follow-up live-animal testing of any positive animals using a combination of tissues (ear biopsy, tonsil and/or rectal biopsy). Using this approach, and based on published RT-QuIC sensitivity and specificity estimates, we estimate an overall probability of detecting a CWD positive animal to be 76% to 94% and a probability of detecting true-negatives at 99% to 100%. Given that zero live-animal testing for CWD is currently performed in Minnesota, the RT-QuIC–based approach described in HF1202/SF1526 would provide the state

with an unprecedented opportunity to proactively detect CWD-positive animals, perhaps months or years earlier than traditional post-mortem testing. It's important to note that this approach would supplement, not replace, regulatory CWD testing for CWD surveillance. Moreover, concerns relating to false-positives are minimized under the testing proposed in HF1202/SF1526, as the probability that a deer not infected with CWD will test positive (i.e., false-positive diagnosis) is expected to be 1% or less.

At this time management or regulatory decisions should not be based *SOLELY* on data generated from RT-QuIC. However, RT-QuIC has been repeatedly and independently shown to perform well as a diagnostic test for CWD, generating accurate information. The utility of such a tool should not be disregarded. In light of well-documented cases of human-mediated spread of CWD through both live animals and carcasses, we believe that RT-QuIC can be effectively used as a screening tool that can prevent the unintentional spread of CWD. Implementation of RT-QuIC in parallel to existing regulatory CWD testing would provide state, tribal, and federal agencies with expanded options to proactively fight CWD.

Assessment of Cervid Genetics and Susceptibility to CWD

During the current legislative session, there have been statements regarding the breeding of white-tailed deer to make them genetically "resistant" or even "immune" to CWD. We note that **none** of the white-tailed deer genotypes documented to date are known to prevent CWD infection.[18–21] Therefore, it is currently presumed that all white-tailed deer are vulnerable to infection with CWD, regardless of their genetic background.

Research has demonstrated that certain genetic factors can influence traits such as when and how prion diseases manifest within infected hosts. With respect to CWD, there are select genotypes associated with characteristics such as extended incubation periods and prolonged survival or the distribution of prions across infected host tissues.[18,20,21] An important note is that the genotypes shaping these traits often differ between cervid species (e.g., white-tailed deer, mule deer, elk, caribou). The major takeaway from these studies is that all cervids—regardless of species and genotype—can still become infected with CWD prions.[18,20–22]

In various captive deer and elk facilities, selective breeding continues to be used as a prospective management option.[19,23,24] In general, this involves genetic sequencing of the herd and prioritizing mating pairs that would produce offspring with genotypes considered "favorable" in respect to CWD (e.g., prolonged incubation periods, longer survival). And while this approach appears capable of shifting herd genetics over time (i.e., larger share of cervids expressing "preferred" genotypes), it is unclear what effect—if any—this has on CWD management.[19] At this time, there are no peer-reviewed, published data clearly demonstrating that selective breeding of deer can prevent the transmission of CWD.

Several unresolved concerns that relate to selective breeding for CWD "resistance" must be

considered. One is the possibility that any deer with a "resistant" genotype that lengthens CWD's incubation period (e.g., ~2 years between infection and death vs. 5 years) could potentially shed infectious prions into the environment for a longer period.[21,22] Consequently, these animals might have increased capacity to spread the disease compared to deer with shorter incubation periods. Thus, selectively breeding deer to promote an extended incubation period could actually promote CWD transmission.

The observation that genetically "resistant" deer can still be infected with CWD and shed prions into the environment for longer periods is critical when comparisons are raised between the management of scrapie (a prion disease of sheep and goats) and the management of CWD of captive cervids. Whereas the vast majority of scrapie management practices in the USA occur entirely within farm settings, CWD management must occur in both wild and captive environments. Dense populations of wild deer that surround captive deer facilities are both (1) potential sources of CWD transmission to captive deer (regardless of genotype) and (2) susceptible to CWD prions originating from captive deer (regardless of genotype). For these reasons, live-animal testing of captive deer herds must be established alongside any CWD-related breeding program.

In addition, there are concerns that selecting genotypes deemed "favorable" for CWD may, at the same time, cause disadvantages in other areas that contribute to deer survival. Helping fuel this notion is an apparent scarcity of CWD "resistant" genotypes in many free-ranging deer populations. And although certain confounding factors have likely had some impact on shaping overall genetic distribution, it also remains plausible that these CWD "resistant" genotypes are rare in wild deer populations because they reduce overall fitness and have been evolutionarily selected against.

Finally, any discussion surrounding CWD-related deer breeding programs must also include a discussion of CWD prion strain variation. Several strains of CWD prions are known to circulate in deer, and it is likely that there are many undiscovered strains circulating in CWD-positive deer and elk herds across the USA.[25–28] The scientific community does not yet know how selective breeding practices for CWD "resistant" deer would affect CWD prion strain variation. A concern is that captive deer having CWD "resistant" genotypes would ultimately promote the production of new CWD prion strains.[29,30] These new CWD prion strains could have unique properties that alter species barriers (e.g., could infect livestock, wildlife, humans) and could prevent traditional antibody diagnostic tests (e.g., ELISA and IHC) from working as expected (i.e., the antibodies used by these tests might not work with emerging CWD prion strains).[19,28]

Although the scientific literature clearly shows that deer genetics can influence CWD disease progression at the individual level, no genetic background carries 100% protection against CWD. While research in this area is ongoing, the breeding of deer that are "resistant" to CWD is not a silver bullet for managing the disease and has important risks that must be considered.

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