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May 3, 2021

VIA ELECTRONIC FILING

The Honorable James R. Mortenson
Administrative Law Judge
Office of Administrative Hearings
600 North Robert Street
PO Box 64620
St. Paul, MN 55164-0620

Re: In the Matter of the Proposed Exempt Rules Governing Farmed Cervidae, Minnesota Board of Animal Health, Request for Review and Approval of Exempt Rules Under Minnesota Statutes, Section 14.388, Revisor's ID Number R-04699; *Public Comment of Minnesota Elk Breeders Association Opposing Use of the Good Cause Exemption for Rule Amendment*

Dear Judge Mortenson:

I write on behalf of the Minnesota Elk Breeders Association to oppose the Minnesota Board of Animal Health's ("Board") request for review and approval of its use of Minn. Stat. § 14.388's good cause exemption to circumvent statutorily-required rulemaking procedures in amending Minn. R. 1721.0420, Subp. 3.

Critically, the question before you in this truncated rulemaking proceeding is not whether the Board's proposed rule amendment is wise, it is not. The question before you is likewise not whether the Board's proposed rule amendment would be justified after the full-panoply of rulemaking procedures, including full public notice, comment, and hearing, have been exhausted, it would not. The only question in this proceeding is whether the Board has met its extremely high burden of establishing that the circumvention of statutorily-prescribed rulemaking procedures and protections is warranted. But the Board's conclusion that its proposed rule would address a "serious and immediate threat to the public health, safety, and welfare" is based on nothing more than conjecture, assumptions, and incomplete investigations. Speculation and guess are not enough to satisfy the Board's high burden. Moreover, the Board makes no effort to explain how any delay resulting from the use of statutorily-prescribed rulemaking would harm the public interest and thus

has likewise not satisfied its high burden of establishing such harm to exempt it from Minn. Stat. ch. 14's rulemaking procedures.

Both the Minnesota Court of Appeals and the Office of Administrative Hearings ("OAH") have held that an agency's use of exempt rulemaking must be strictly scrutinized because it further deprives the agency of a full record upon which rulemaking determinations will be made, and deprives the public of agency accountability and the opportunity to be fully heard, before adopting rules that may impact people's lives and livelihoods. Those decisions thus held that approval of the use of Minn. Stat. § 14.388's good cause exemption must be withheld in all but the most exceptional of circumstances and must be approved only in the case of true emergencies. This is not such an exceptional case and there is no true and immediate emergency. The Board's request for the approval of its rule amendment under Minn. Stat. § 14.388 must therefore be denied.

A. Applicable Law Governing Requests for Approval of Exempt Rulemaking Under Minn. Stat. § 14.388

An "agency shall adopt, amend, suspend, or repeal its rules in accordance with the procedures specified in [Minn. Stat. §§] 14.001-14.69, and only pursuant to the authority delegated by law and in full compliance with its duties and obligations." Minn. Stat. § 14.015. The procedures set forth in the Minnesota Administrative Procedure Act are intended to, among other things, "provide oversight of powers and duties delegated to administrative agencies . . . ensure a uniform minimum procedure . . . [and] increase public participation in the formulation of administrative rules." Minn. Stat. § 14.001. These procedures include, among other things, the preparation by the agency of a complete statement of the need for and reasonableness of the rule (SONAR) and the publication of that SONAR for public review (*see, e.g.*, Minn. Stat. § 14.131 & 14.23), a 30-day public comment period (*see, e.g.*, Minn. Stat. § 14.22), and the holding of a public hearing (*see, e.g.*, Minn. Stat. § 14.25).

The Minnesota Court of Appeals has recognized that by circumventing these procedures, exempt rulemaking "obviates the opportunity to bring to the agency's attention all relevant aspects of the proposed action and thereby enhance the quality of agency decisions . . . [and] has a negative impact on the statutory goal of increasing public accountability of administrative agencies." *Jewish Cmty. Action v. Comm'r of Pub. Safety*, 657 N.W.2d 604, 610 (Minn. App. 2003) (internal quotation and citation omission). It is for this reason that in invalidating a rule amending driver's license application requirements to address the threat of terrorism in the wake of 9/11 under Minn. Stat. § 14.388's good cause exemption, the Minnesota Court of Appeals held that "exempt rulemaking is an exceptional procedure and is reserved for emergencies." *Id.* (citing *Buschmann v. Schweiker*, 676 F.2d 352, 357 (9th Cir. 1982)) (emphasis added).

The Office of Administrative Hearings, too, has recognized that an agency's use of Minn. Stat. § 14.388 to circumvent administrative rulemaking procedures should be approved of in only exceptional circumstances. As Judge Case held in disapproving of the use of the good cause

exemption in *In re Exempt Permanent Rule Relating to Environmental Review of Recreational Trails*, OAH 82-9008-32965, 2015 WL 9459861, at *2 (Minn. Off. Admin. Hrgs. Dec. 2, 2015):

> Under the good cause exemption, both the Board's rulemaking powers and the breadth of the review by the Office of Administrative Hearings are sharply reduced . . . because the good cause exemption, by its terms, contemplates that administrative rules will only be promulgated pursuant to this method in order to meet truly exigent circumstances.

Id. (emphasis added). Minn. Stat. § 14.388 should therefore "be used sparingly and rules proposed through the expedited process must be strictly scrutinized." *Id.; see also In re Adopted Exempt Permanent Rule of the Minnesota Pollution Control Agency Governing Municipal Effluent Limitations*, OAH 19-9003-34654, 2017 WL 5662794, at *4 (Minn. Off. Admin. Hrgs. Oct. 31, 2017).

Thus, to obtain approval of its use of Minn. Stat. § 14.388's good cause exemption, the Board bears a high burden of establishing "(1) that there is a serious and immediate threat to public health, safety, or welfare; (2) that the rules address that threat; and (3) that it would be contrary to the public interest to follow usual rulemaking procedures." *Jewish Cmty. Action*, 657 N.W.2d at 608 (citing Minn. Stat. § 14.388). And, "[t]o satisfy the contrary-to-public-interest factor, the [Board] must reasonably quantify the delay that will occur if formal rulemaking is undertaken; show with reasonable particularity how the public interest will likely be harmed by that delay; and demonstrate specifically how the exempt rulemaking procedure will better serve the public interest." *Id.*

The Board's reliance on *In re Proposed Exempt Rules of the Minnesota Racing Commission Governing Horse Racing*, OAH 10-9011-33393, 2016 WL 3029803 (Minn. Off. Admin. Hrgs. May 4, 2016), does not change the analysis because that case is distinguishable in several respects. First, there was notably no opposition to the rule at issue in that case. *Id.* Here, the Minnesota Elk Breeders Association vehemently opposes the adoption of the proposed rule. Moreover, the emergency rule adopted in *Minnesota Racing Commission* was intended to stop the spread of a highly contagious disease among race horses. *Id.* In contrast, CWD is not highly-contagious and, in fact, has a slow infection rate in the wild. *See, e.g.*, **Attach. J.** Further, the rule at issue in *Minnesota Racing Commission* was adopted to address a confirmed issue – namely, the transmission of this highly contagious disease among race horses.[1] As discussed below, the alleged issue here – namely, the spread of CWD between wild cervidae and farmed cervidae despite the existence of exclusionary fencing – is far from confirmed. Indeed, the Board has admitted that its investigation is not yet complete. The rule at issue here, therefore, unlike the rule at issue in *Minnesota Racing Commission*, is being proposed based upon nothing more than incomplete investigations, assumptions, and speculation. Critically, the use of Minn. Stat. § 14.388's good cause exemption must be evaluated on a case-by-case basis. That the Minnesota Racing

[1] *See, e.g.*, https://www.leg.mn.gov/archive/sonar/SONAR-04443.pdf at 17.

Commission met its extremely high burden in an earlier case does not negate the fact that the Board has failed to meet that burden here.

B. <u>The Board's Requested Amendment and its Impact on Minnesota Elk Breeders</u>

Minn. R. 1721.0370-.0420, adopted pursuant to Minn. R. ch. 14's ordinary rulemaking procedures,[2] impose certain rules and regulations on owners of farmed cervidae herds – including, among other animals, deer and elk. Minn. R. 1721.0410, Subp. 5 restricts the movement of farmed cervidae from a designated chronic wasting disease (CWD) endemic area (defined to include "a geographic area as determined by the board where CWD is present in wild cervidae populations") to other locations throughout the State of Minnesota. But, as currently written, Minn. R. 1721.0420, subp. 3 has, since 2013, excluded from a CWD endemic area farmed cervidae if:

> A. the herd has been maintained in such a way that commingling of farmed Cervidae and wild Cervidae has been prevented for a continuous period of time that began no later than 180 days following the initial designation of the area as CWD endemic; or
>
> B. the herd has been maintained in such a way that commingling of farmed Cervidae and wild Cervidae has been prevented for at least 36 consecutive months.

In the SONAR prepared for the adoption of this rule, the Board concluded that "[t]his is reasonable because the specification of ten miles for the radius of a CWD endemic area and the criteria for exclusion of individual herds was developed with full support from the Board's Farmed Cervidae Advisory Committee. Experts from the Board, the Minnesota Department of Natural Resources, the University of Minnesota, the United States Department of Agriculture, and all producer groups agree that these criteria are appropriate for the protection of the health of Minnesota farmed and wild cervidae."

The Board now, without providing in this proceeding for fulsome input from the groups involved in the rule's initial drafting and enactment – *i.e.*, the Minnesota Department of Natural Resources, the University of Minnesota, the United States Department of Agriculture, and cervidae producer groups[3] – seeks the removal of this exclusion and the expansion of a designated endemic

[2] *See* https://www.leg.mn.gov/archive/sonar/SONAR-03976.pdf.

[3] In fact, the Board's conduct in this case appears purposefully tailored to prevent producer input regarding the use of Minn. Stat. § 14.388 to adopt this rule amendment. For example, in an April 16, 2021 email to the members of the Farmed Cervidae Advisory Committee, which includes members of the Minnesota Elk Breeders Association, the Board expressly stated that at its April 21, 2021 meeting "we will be providing the board members with an update on our farmed Cervidae rulemaking but will **<u>not</u>** be asking the board members to vote on and approve a rules draft at this meeting." **Attach. B**. And in the agenda for the April 21, 2021 Board meeting in which the use of Minn. Stat. § 14.388's good cause exemption was authorized, no reference is made to its potential

area from 10 miles to 15 miles.[4] The Board's amendment would deal a devastating blow to the farmed cervidae industry generally and the elk farming industry specifically – an industry which notably has not had a case of CWD since 2009. **Attach. A**. For example, there are currently 13 cervidae farms approved by the Board for exclusionary fencing and intrastate movement from a designated endemic zone. *Id.* The removal of this exception will result in great economic harm to these businesses and others in the future which, despite their use of expert-approved precautionary measures to avoid the transmission of CWD, and despite any conclusive scientific data establishing that CWD is being transmitted between wild and farmed cervidae, would indefinitely restrict farmers within a designated CWD endemic zone from selling their herd outside that zone other than for slaughter. *Id.* This could result in the shuttering of many of these farms – and in turn the loss of these Minnesota farmers' livelihoods. *Id.*

Given the potential impact of this rule, robust rulemaking procedures, with input from experts and interested parties, must be followed before any rules are adopted or amended.

C. The Board's Failure to Satisfy its High Burden of Demonstrating a Serious and Immediate Threat to Public Health, Safety, or Welfare

To obtain approval for its use of Minn. Stat. § 14.388's good cause exemption, the Board must first establish that the rule it proposes will address a "serious and immediate threat to public health, safety, or welfare."

The alleged "serious and immediate threat" identified by the Board in its April 26, 2021 letter is that "allowing any farmed Cervidae to move from a CWD endemic area to another area in the state is not reasonable and presents a very serious and immediate threat to Minnesota's public health, safety, or welfare." The basis for this conclusion is the Board's assertion that there is

use. **Attach. C**. Moreover, following the April 21, 2021 Board meeting, the Minnesota Elk Breeders Associations requests for information have been stonewalled by the Board. *See, e.g.*, **Attach. D.**

[4] The Minnesota Elk Breeder's Association opposes both the removal of this exclusion and the CWD endemic area expansion from 10 to 15 miles. This comment letter, however, focuses on the removal of the endemic area exclusion for cervidae farms that prevent comingling because the Board in its April 26, 2021 letter appears to agree that its rule amendment to expand the CWD endemic area does not meet the requirements of Minn. Stat. §14.388. That is, the Board admits that this proposed amendment simply formalizes that which has been its practice since March 2019. Assuming the Board would continue this practice during formal rulemaking, there is certainly no "serious and immediate threat to public health, safety, or welfare" such that the public interest requires emergency rulemaking exempt from statutory procedure.

evidence that CWD transmission from wild Cervidae in Winona County to a double-fenced farmed cervidae herd in Winona County occurred sometime before September of 2019.[5]

The purported "evidence" provided by the Board, however, cannot support the use of Minn. Stat. § 14.388 to circumvent statutory rulemaking requirements. In fact, the Board provides no actual evidence that any such transmission ever occurred. Instead it offers only assumptions and speculation in support of its emergency rulemaking. And in deciding whether to approve of the use of exempt rulemaking, the Board's conclusions and determinations must be strictly scrutinized.

The Board's support for its conclusion that there was a wild cervidae to farmed cervidae transmission in Winona County sometime before September of 2019 is essentially as follows: (1) since at least early 2019, CWD infection was detected in wild cervidae (specifically deer) in Winona County; (2) animals from a specific herd of double-fenced (and thus exempt from endemic area) farmed cervidae (specifically deer) were sold to a herd in Houston County in October 2019 and to a herd in Beltrami County in September 2019; (3) one year later, in October 2020, one of the animals sold to the Houston County herd died and tested positive for CWD; (4) one and a half years later, in April 2021, one of the animals sold to the Beltrami County herd died and tested positive for CWD; and (5) the Board claims that it is unaware of any compliance issues at either the Houston County herd or the Beltrami County herd and thus the CWD transmission must have come from Winona County. The Board's reasoning that these facts justify the use of the exceptional and sparingly authorized good cause exemption to rulemaking is flawed in several respects.

First, while the entirety of the Board's conclusion that there is "a serious and immediate threat to public health, safety, or welfare" stems from its determination that CWD infection in Houston County and Beltrami County herds originated from the Winona County herd, there has to-date been no diagnosis of any cases of CWD at the Winona County farm from which those animals were sold. That is, no animal that has died at the Winona County farm at issue has tested positive for CWD. **Attach. A**; April 21, 2021 Meeting (recording available at https://www.bah.state.mn.us/board-members/) at 2:09-2:36. It necessarily follows that there has likewise been no determination that such non-existent CWD infection in that herd was transmitted by wild cervidae in Winona County. Indeed, the Board itself states only that "*[c]urrently*, the primary risk factor for exposure of the Winona County herd to CWD is from CWD infection in wild deer outside the fence." (Emphasis added.) In other words, while the Board seeks to avoid rulemaking to impose this emergency rule based upon the alleged transmission of CWD from wild cervidae in Winona County to double-fenced farmed cervidae in Winona County, there has been

[5] Notably, "public" necessarily refers to "people" but the Board does not argue that CWD is transmissible to humans – indeed it concedes just the opposite. The alleged threat of animal-to-animal transmission thus does not meet the requirement that there be a "serious and immediate threat to public health, safety, or welfare."

(1) no diagnosis of CWD infection in that Winona County herd, and (2) no final determination that such infection originated with wild cervidae in Winona County.

Second, while the Board asserts that the Houston County and Beltrami County cases were found at farms that were fully-compliant with CWD regulations and thus transmission must have come from the Winona County herd, the facts call into question this conclusion. Notably, the Board has admitted that it has not yet completed its investigation into the Beltrami County herd and it has not provided its written findings as to the Houston County herd. **Attachs. A & E**. The conclusion that CWD transmission could have only originated from the Winona County herd (particularly in light of the fact that no CWD has been detected in that herd) is, therefore, premature.

Moreover, the information provided to-date regarding the Beltrami County herd suggests potential non-compliance with applicable Minnesota rules. Notably, Minn. R. 1721.0420, Subp. 1.D. requires that farmed cervidae that die or are slaughtered "must be tested with an official CWD test" and that "[s]amples to be tested for CWD must be submitted to a laboratory approved by the board within 14 days of sample collection." (Emphasis added.) But at an April 21, 2021 meeting of the Board, it was reported that (1) one deceased animal at the Beltrami County site was too decomposed to test – which establishes that requisite testing rules were not complied with – and (2) samples from five additional animals that died at the site this past winter were still at the lab waiting to be tested – suggesting that the 14-day sample collection requirement was likewise not complied with for these five animals. April 21, 2021 Meeting at 2:09-2:36; **Attach. A**. Further, the original source of these latter five animals has not been disclosed, suggesting that there is yet another potential source for CWD transmission. **Attach. A.**

Finally, the Board suggests that imposing its draconian solution to a problem it has not yet conclusively determined exists must happen now without statutorily-required procedural safeguards and processes because there is no way to test live cervidae for CWD. Thus, it asserts, there is no other way to mitigate the risk of intrastate spread by farmed animals. But that, too, is not accurate. There is, in fact, available testing for live animals that utilize blood, feces, saliva, and rectal tissue that have been shown in studies to be highly useful in detecting CWD in live animals. **Attachs. F-H;** April 21, 2021 Meeting at 1:09-2:10. These very tests are being utilized in other states, for instance Texas, to control the spread of CWD in the intrastate transfer of farmed cervidae. *See, e.g.*, **Attach. I.** While the Board has resisted efforts to utilize these live testing tools and thus has not yet approved of these tests, its assertion that there exists no other way to address this issue is simply not true.

D. The Board's Failure to Satisfy Its Burden of Quantifying the Delay Imposed by Formal Rulemaking and Establishing How the Public Interest will be Harmed by that Delay

Before an agency's request for approval of its use of Minn. Stat. § 14.388's good cause exemption can be granted, it "must reasonably quantify the delay that will occur if formal

rulemaking is undertaken; show with reasonable particularity how the public interest will likely be harmed by that delay; and demonstrate specifically how the exempt rulemaking procedure will better serve the public interest." *Jewish Cmty. Action*, 657 N.W.2d at 608. The Board has failed entirely to even try to satisfy this requirement.

Notably absent from the Board's April 26 letter is any quantification, let alone reasonable quantification, of the delay that will occur if formal rulemaking is undertaken to amend Minn. R. 1721.0420, Subp. 3. This is particularly relevant in light of the fact that the Board is currently in the process of formal rulemaking. Indeed, it began that process nearly a year ago, with a request for public participation and written comments on the rulemaking process issued on June 29, 2020, and the first meeting of the Farmed Cervidae Advisory Committee held on August 26, 2020. The process of proposing, drafting, and revising rule amendments continues to this very day. **Attach. A.** In fact, the surprise announcement of the Board that approval of emergency rulemaking would be sought on April 26 was made at an April 21 meeting that provided updates on this formal rulemaking process. The Board's request for approval must be denied under applicable Minnesota law for this reason alone. *See Jewish Cmty. Action*, 657 N.W.2d at 610 (invalidating rule and holding that even agency believed some delay was okay because formal rulemaking had previously been initiated to adopt rule before being suspended).

Further, the Board likewise does not provide any argument, let alone support sufficient to meet its burden for any such argument, that the public interest will be harmed by this unarticulated delay. Instead, the Board argues only for the public interest that it claims will ultimately be served by the adoption of its proposed rule amendment. But whether a rule that is eventually adopted will serve the public interest is irrelevant to whether the public interest will be harmed by a quantifiable delay (unarticulated here) caused by following the statutorily-required rulemaking process. And an argument that the sooner a rule is adopted the better is not enough. Indeed, such an argument could be made in support of abandoning the rulemaking process in every situation, rendering formal rulemaking and the rulemaking procedures required by the Minnesota legislature obsolete. Such an argument is not sufficient under Minnesota law. Regardless of whether one believes that the Board's ultimate public interest goals are admirable, they are not sufficient to approve of its use of Minn. Stat. § 14.388's good cause exemption to formal rulemaking. This is particularly true when, as is the case here, the alleged "serious and immediate threat to public health, safety, or welfare" is far from established.

Because the Board has failed to establish that the requirements under Minnesota law for approval of a rule under Minn. Stat. § 14.388's good cause exemption have been met, its request for approval of its amendments to Minn. R. 1721.0420, subp. 3 must be denied.

Sincerely,

Taft Stettinius & Hollister LLP

/s/ Maren M. Forde

Maren M. Forde

MMF:amw

DECLARATION OF BRENDA HARTKOPF

I, Brenda Hartkopf, declare the following:

1. I am the Executive Secretary of the Minnesota Elk Breeders Association, a member of the Minnesota Board of Animal Health's Farmed Cervidae Advisory Committee, and an Elk producer in Minnesota.

2. In my opinion and experience, CWD detection in a wild cervidae within 15 miles of my herd without the ability to meet Minnesota's current Minn. R. 1721.00420 exemption from a CWD endemic zone by the use of exclusionary fencing would result in an indefinite income loss of at least 75%. This income loss would result in me going out of business.

3. This income loss would be similarly incurred by any other farmed cervidae business in such a circumstance.

4. The Board of Animal reported on April 21 that there are currently 13 cervidae farms approved by the Board for exclusionary fencing and intrastate movement.

5. The elk farming industry has not had a case of confirmed CWD since 2009.

6. There has been no diagnosis of CWD at the Winona County farm at issue.

7. The investigation into the source of CWD infection in the Beltrami County herd has not been concluded and the investigation into the source of CWD infection in the Houston County herd at issue has not been made public.

8. The Board of Animal Health reported that one deceased animal at the Beltrami County had decomposed beyond the ability to test tissues and that five animals that died this past winter had just been recently sampled for CWD testing. The original source of these five animals has not been disclosed.

9. The drafting, review, and consideration of amendments to Minn. R. ch. 1721 through the formal rulemaking process is ongoing.

Dated: May 3, 2021

Brenda Hartkopf

Signed: Wright County, Minnesota

Attach. A

From: Balghiti, Annie (BAH) <Annie.Balghiti@state.mn.us>
Sent: Friday, April 16, 2021 4:35 PM
To: Rich Meech <rstrr2003@gmail.com>
Cc: steve.notch@co.stearns.mn.us; kle1019@live.com; dan.miller@riverland.edu; dan@danbmiller.com; aacordry@gmail.com; jihnen@yahoo.com; Carstensen, Michelle (DNR) <michelle.carstensen@state.mn.us>; Anderson, Kelly (MDA) <kelly.anderson@state.mn.us>; Scheftel, Joni (MDH) <joni.scheftel@state.mn.us>; craig.engwall@mndeerhunters.com; MarkL@luckylandelk.com; info@mneba.org; brenda.hartkopf@gmail.com; dysimon@livereindeer.com; miles@glifwc.org; pkebec@glifwc.org; Stephan.L.Schaefbauer@usda.gov; Torrison, Jerry (UofM) <torri001@umn.edu>; rmillertodd@gmail.com; Glaser, Linda (BAH) <linda.glaser@state.mn.us>; Wheeler, Courtney (BAH) <courtney.wheeler@state.mn.us>; Thompson, Beth (BAH) <beth.thompson@state.mn.us>; Crusan, Michael (BAH) <michael.crusan@state.mn.us>
Subject: RE: Farmed Cervidae Advisory Committee, Updates

Good afternoon,

At Wednesday's board meeting, we will be providing the board members with an update on our farmed Cervidae rulemaking but will **not** be asking the board members to vote on and approve a rules draft at this meeting.

When we are ready to present a rules draft to the board members for approval at a board meeting, we will notify and provide that rules draft to the Advisory Committee members in advance of that meeting.

Attach. B

Please note: When we do ask the board members to approve a rules draft at a meeting, the rules draft that we ask the board members to approve are **not the final rules**. The draft we ask them to approve is the draft we would publish, which opens the second public comment period.

There is no **final proposed** draft of the rules until we publish a proposed rules draft with the Notice of Intent to Adopt in the State Register. This is the next formal step in the rulemaking process. When the Board publishes this Notice of Intent to Adopt, (1) the rules are formally proposed, and (2) the public has a second opportunity to comment on the rules. Public comment means comments from anyone outside of our agency. The Board again reviews and considers all public comment it receives during this formal comment period. The comments received during this phase of the rulemaking are reviewed by the Administrative Law Judge when the Board submits its rulemaking record for review.

There is no **final** rules draft until the end of the rulemaking process when the Board publishes its Notice of Adoption and the final rules draft. There are several steps in the rulemaking process that must be completed before we reach that stage.

Let us know if you have any questions.

Have a good weekend, all!
Annie

Annie Balghiti, JD |State Program Administrator Senior
Minnesota Board of Animal Health
625 Robert St. N
St Paul, MN 55155
O: 651-201-6805
C: 612-209-4729
www.mn.gov/bah

MINNESOTA
BOARD OF ANIMAL HEALTH

From: Rich Meech <rstrr2003@gmail.com>
Sent: Thursday, April 15, 2021 8:57 AM
To: Balghiti, Annie (BAH) <Annie.Balghiti@state.mn.us>
Cc: steve.notch@co.stearns.mn.us; kle1019@live.com; dan.miller@riverland.edu; dan@danbmiller.com; aacordry@gmail.com; jihnen@yahoo.com; Carstensen, Michelle (DNR) <michelle.carstensen@state.mn.us>; Anderson, Kelly (MDA) <kelly.anderson@state.mn.us>; Scheftel, Joni (MDH) <joni.scheftel@state.mn.us>; craig.engwall@mndeerhunters.com; MarkL@luckylandelk.com; info@mneba.org; brenda.hartkopf@gmail.com; dysimon@livereindeer.com; miles@glifwc.org; pkebec@glifwc.org; Stephan.L.Schaefbauer@usda.gov; Torrison, Jerry (UofM) <torri001@umn.edu>; rmillertodd@gmail.com; Glaser, Linda (BAH) <linda.glaser@state.mn.us>; Wheeler, Courtney (BAH) <courtney.wheeler@state.mn.us>; Thompson, Beth (BAH) <beth.thompson@state.mn.us>; Crusan, Michael (BAH) <michael.crusan@state.mn.us>
Subject: Re: Farmed Cervidae Advisory Committee, Updates

Attach. B

Good Morning ,

Is the plan to present the final rules draft for approval in next week's quarterly board meeting ?

If so, will the advisory board members see the final draft before the meeting ?

Thanks,

Rich Meech

President Minnesota Deer Farmers Association

On Mon, Feb 22, 2021 at 1:27 PM Balghiti, Annie (BAH) <Annie.Balghiti@state.mn.us> wrote:

Good afternoon, committee members,

Attached is an updated proposed draft of the rules (dated 2.19.21), amended based in part on last week's listening sessions the Board held for the farmed Cervidae industry. If you have feedback on this draft, please "reply all" and send us your comments.

Minutes from the January 22 advisory committee meeting are now posted on our website. We will publish the attached proposed rules draft on our website sometime today.

FYI, we are giving a rulemaking presentation to update our board members at our quarterly board meeting this Wednesday, February 24. We will present this rules draft at the board meeting but will **not** be asking the board members to approve this draft as the final rules draft. Our rulemaking presentation will be to discuss the proposed amendments to date and to answer board members' questions.

We (Dr. Glaser, Dr. Wheeler, and I) are also planning to draft an advisory committee report in the coming weeks, to summarize the work of the advisory committee. We will circulate a draft of that report to you all once it is available.

Please let us know if you have any questions.

Thank you,

Annie

Attach. B

Annie Balghiti, JD |State Program Administrator Senior

Minnesota Board of Animal Health

625 Robert St. N

St Paul, MN 55155

O: 651-201-6805

C: 612-209-4729

www.mn.gov/bah

MINNESOTA
BOARD OF ANIMAL HEALTH

MINNESOTA
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Agenda: April Board Meeting

Wednesday, April 21, 2021 | 9:30 a.m.

Remote Access: Online: Join the meeting on Microsoft Teams
Call in: 1+651-395-7448, Conference ID: 217223400#

9:30 a.m.	**Call Meeting to Order (5 mins)** Notice of Meeting and Resolution Approval of Minutes from February 24, 2021 Approval of Agenda	**Dean Compart**
	Board of Animal Health Update	Beth Thompson
	Legislative Update	Beth Thompson
	USDA Update	Stephan Schaefbauer
	College of Veterinary Medicine Update	Laura Molgaard
	Veterinary Diagnostic Laboratory Update	Jerry Torrison
	CWD Research Update	Peter Larsen
	Farmed Cervid Rule Making	Linda Glaser, Annie Balghiti
	H6N1 Low Pathogenic Avian Influenza Update	Dale Lauer
	COVID-19/MDH Update	Joni Scheftel
	Appointment of Board Leadership	Board Members
	CLOSED SESSION: Appointment of Executive Director	
1:30 p.m.	**Adjourn**	

Attach. C

[redacted]

From: Glaser, Linda (BAH) <linda.glaser@state.mn.us>
Sent: Wednesday, April 28, 2021 9:16 AM
To: Brenda Hartkopf <info@mneba.org>
Subject: Automatic reply: Questions for You

I am out of the office and will return on Monday, 5.3.2021.

I will respond to your email when I return to the office. For immediate assistance with the farmed cervid program please contact the program staff at farmed.cervidae@state.mn.us or the Farmed Cervidae phone line at 651-201-6804.

Attach. D

[redacted]

From: Balghiti, Annie (BAH) <Annie.Balghiti@state.mn.us>
Sent: Wednesday, April 28, 2021 1:41 PM
To: Thompson, Beth (BAH) <beth.thompson@state.mn.us>; info@mneba.org
Cc: Glaser, Linda (BAH) <linda.glaser@state.mn.us>; Wheeler, Courtney (BAH) <courtney.wheeler@state.mn.us>
Subject: RE: Questions for You

Yes, thank you, Dr. Thompson.

Please submit a data request for the remaining data you are requesting.

Thank you,

Annie Balghiti, JD |State Program Administrator Senior
Minnesota Board of Animal Health
625 Robert St. N
St Paul, MN 55155
O: 651-201-6805
C: 612-209-4729
www.mn.gov/bah

MINNESOTA
BOARD OF ANIMAL HEALTH

From: Thompson, Beth (BAH) <beth.thompson@state.mn.us>
Sent: Wednesday, April 28, 2021 12:59 PM
To: info@mneba.org
Cc: Glaser, Linda (BAH) <linda.glaser@state.mn.us>; Balghiti, Annie (BAH) <Annie.Balghiti@state.mn.us>; Wheeler, Courtney (BAH) <courtney.wheeler@state.mn.us>
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I'm copying Annie Balghiti to have her respond to the portion of your question on animal origin and movement information as I believe that may need to be requested through a data request.

Attach. D

Beth

Beth S. Thompson, JD, DVM
Executive Director | State Veterinarian
Minnesota Board of Animal Health
625 Robert St. N
St Paul, MN 55155
O: 651-201-6844
C: 507-272-6254
www.mn.gov/bah

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BOARD OF ANIMAL HEALTH

From: Brenda Hartkopf <info@mneba.org>
Sent: Wednesday, April 28, 2021 9:16 AM
To: Thompson, Beth (BAH) <beth.thompson@state.mn.us>
Cc: Glaser, Linda (BAH) <linda.glaser@state.mn.us>
Subject: FW: Questions for You

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1. When did the five animals with CWD results pending at NVSL die and why are the samples only now being tested?
2. Are these samples from remains or were the samples harvested when the animals died and just not sent in yet?
3. Where did these five animals originate from and when did they join the herd?

It seems like there was an extreme amount of death loss over the winter months in this herd.

Thanks,
Brenda

Brenda Hartkopf
Minnesota Elk Breeders Association
(320) 543-2686
info@mneba.org – www.mneba.org

Attach. D

From: Brenda Hartkopf <info@mneba.org>
Sent: Friday, April 23, 2021 4:09 PM
To: 'Glaser, Linda (BAH)' <linda.glaser@state.mn.us>
Subject: Question for You

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MINNESOTA
BOARD OF ANIMAL HEALTH

Attach. E

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Attach. E

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Minnesota Center for Prion Research and Outreach

Wet lab and field research update: July 2019 - April 2021

Where the research began.

- **July 2019:** MNPRO receives ~$2M (LCCMR and RARF) to launch and develop novel CWD diagnostics.
- **Dec. 2019:** Wetlab is fully outfitted and lab staff are onboarded.
- **Jan. 2020:** First successful RT-QuIC assay is completed.
- **March 2020:** COVID-19 hits and UMN labs shutdown
- **July 2020 to present:** MNPRO lab operating at 50% capacity due to pandemic

How MNPRO has grown.

- Built working relationships with research partners associated with 9 UMN colleges/centers, 7 universities, 18 tribal entities, 4 state agencies, 3 federal agencies.
- Secured 5 additional grants totalling nearly $1M to advance wet lab functionality, ecological understanding of CWD, CWD surveillance in Minnesota tribal lands, diagnostic development, and CWD detection and decontamination in venison processing.
- Expanded RT-QuIC testing capabilities with 4 plate reader machines.

What MNPRO has accomplished.

- Performed more than 10,000 RT-QuIC reactions on cervid tissues, feces, blood; plant material; water.
- Collaborated with MN DNR investigating the efficacy of RT-QuIC to ELISA (see additional document).
- Developed novel RT-QuIC protocol for CWD prion detection in muscle samples.
- Deployed a cutting-edge diagnostic platform at a MN DNR field station that is capable of identifying CWD-causing prions in deer lymph tissues within 24 hours.
- Advanced research on several novel diagnostic tools, 4 provisional patents and commercial partnership.

What MNPRO can do next.

- Environmental research
  - Farm surveillance network - piloting population-level screening through water, soil, and fecal sampling potentially reducing the risk of moving CWD-positive animals.
  - Ecological impact and modeling of CWD transmission and contamination in Minnesota environments.
- On-site research of hunter harvested samples with newly developed detection methods.

Where MNPRO is going.

- Continue prion detection research and development.
- Investigations to understand CWD spread and persistence in the Minnesota environment.
- Supporting prion research of other Minnesota-based scientists and managers.
- Work with Minnesota communities - CWD education and testing.

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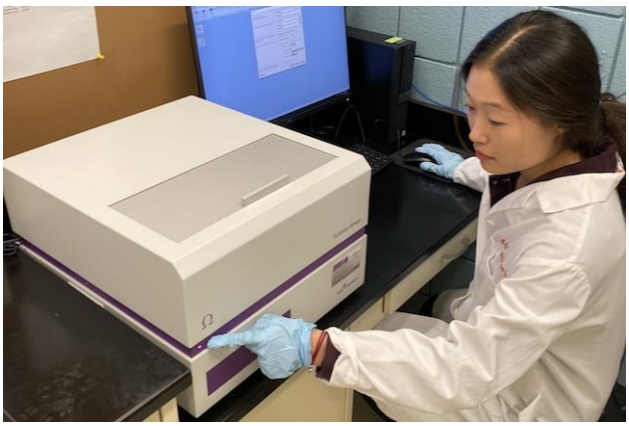
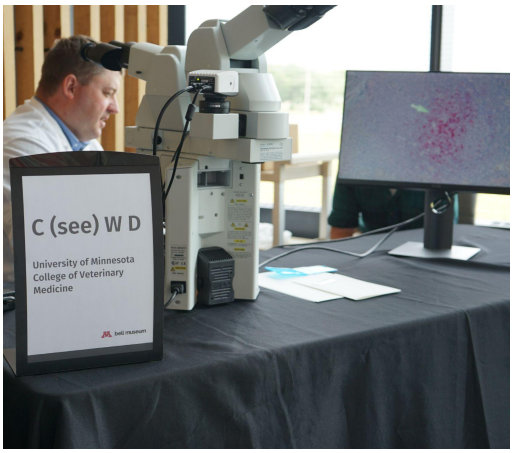
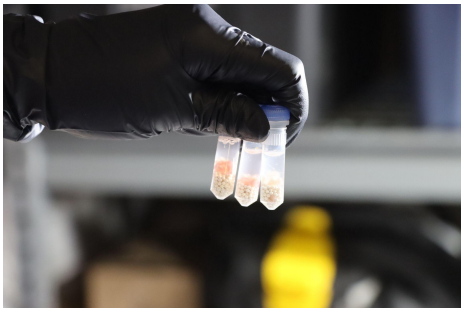
CONTACT: Peter Larsen, PhD
Tiffany Wolf, DVM, PhD
Marc Schwabenlander, MPH

Attach. F




C (see) W D
University of Minnesota
College of Veterinary
Medicine



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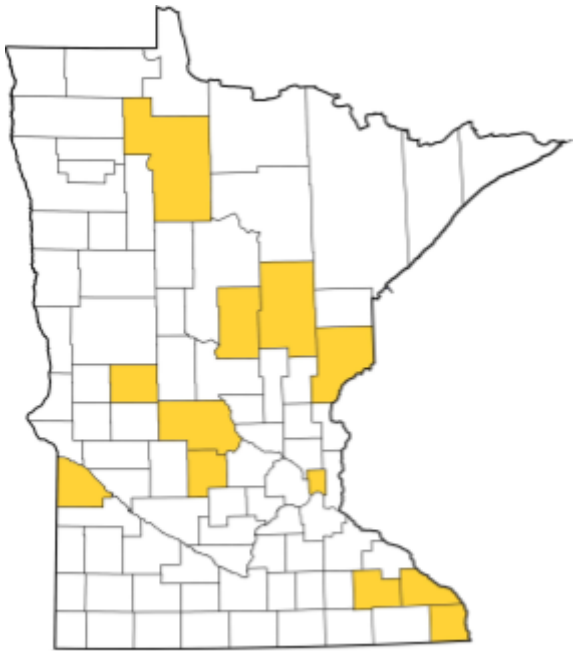
Proposal: Advanced CWD surveillance network

The announcement of a CWD positive cervid farm from Beltrami Co. on April, 7 2021 is a concerning development with respect to the spread of CWD in Minnesota. **Current surveillance measures are inadequate for rapidly detecting and preventing additional spread of CWD throughout the cervid farming industry.** This limitation is directly attributed to the limitations of existing diagnostic tools that are entirely based on post-mortem tissues. Moreover, these tools cannot routinely detect early stage infections. For these reasons, **we are always 2 or 3 years behind the disease**.



Yellow counties with CWD positive farms since 2002.

In 2019, our MNPRO team was awarded approximately $2M by the state (LCCMR and MN Extension RARF) to develop advanced CWD diagnostics with ante-mortem options. As part of this effort, our team has successfully implemented a modern and highly-sensitive test known as RT-QuIC, which can identify CWD-infected deer earlier than current ELISA and IHC protocols. This technology is also capable of surveying environmental samples (soil, plants, feces, water) for the presence of CWD-causing prions.

Our MNPRO team can assist with the formation of a real-time CWD surveillance system that, over time, would reduce or prevent additional farm-to-farm spread of CWD. This advanced surveillance network would help:

1. **stabilize and restore confidence in the cervid-farming industry,**
2. **reduce state and national costs related to CWD,**
3. **protect wild cervid populations across the state.**

The exceptional sensitivity of the RT-QuIC method opens new diagnostic avenues for CWD, including herd-level surveillance. **Active herd-level surveillance is the standard for a large number of diseases that impact agriculturally important species including Johne's disease in cattle, PRRSV and influenza in swine, and Salmonella and avian influenza in poultry.** Surveillance of multiple animals housed within a given environment is achieved through sampling water, surfaces, and feces. Our team proposes to utilize RT-QuIC for herd-level surveillance of cervid farms in Minnesota by using the method to screen soil, feces, and/or water from within farm environments.

Implementation of an advanced CWD surveillance network

Our team recommends a two-year pilot to determine utility of an advanced CWD detection network with the following suggestions:

- a commission be formed to investigate the implementation of a highly-advanced herd-level CWD surveillance network. **This commission should include input by cervid industry leaders, BAH, DNR, and UMN representatives.** The commission would help establish a two-year project to develop best practices for long-term CWD surveillance.
- Year one would focus on securing samples from high-risk farms (i.e., those currently in quarantine and/or trace-outs from positive farms). Samples will be collected twice per year and consist of water, soil, and/or feces representative of the cervid herd.
- **All samples can be blinded to MNPRO researchers to protect farm identity and location from public disclosure (as consistent with current BAH policies). RT-QuIC analyses of environmental samples would be considered as "unofficial results".**
- Putative positive environmental samples would lead to on-farm ante-mortem testing from individual cervids (i.e., blood, saliva, feces) comprising the herd. RT-QuIC positive animals could be culled.
- The BAH would be informed of RT-QuIC results for management and regulatory decisions.
- Environmental sampling and screening can be utilized to assess the efficacy of environmental remediation practices and investigate the terms of quarantine periods on positive farms.

We strongly believe a herd-level RT-QuIC CWD surveillance network will help prevent farm-to-farm spread of CWD, limiting impact across the industry and preventing the long-distance spread of CWD to new geographic areas within the state.

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COMPARISON OF CHRONIC WASTING DISEASE DETECTION METHODS AND PROCEDURES: IMPLICATIONS FOR FREE-RANGING WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) SURVEILLANCE AND MANAGEMENT

Marc D. Schwabenlander[1,5], Gage R. Rowden[1], Manci Li[1], Kelsie LaSharr[2], Erik C. Hildebrand[2], Suzanne Stone[1], Davis M. Seelig[3], Chris S. Jennelle[2], Louis Cornicelli[2], Tiffany M. Wolf[4], Michelle Carstensen[2], and Peter A. Larsen[1]

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[2] Minnesota Department of Natural Resources, 5463 West Broadway, Forest Lake, MN, 55025

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ABSTRACT

Throughout North America, chronic wasting disease (CWD) has emerged as perhaps the greatest threat to wild cervid populations, including white-tailed deer (*Odocoileus virginianus*). White-tailed deer are the most sought after big game species across North America with populations of various subspecies in nearly all Canadian provinces, the contiguous USA, and Mexico. Documented CWD cases have dramatically increased across the white-tailed deer range since the mid-1990s, including in Minnesota. CWD surveillance in free-ranging white-tailed deer and other cervid populations mainly depends upon immunodetection methods (e.g., immunohistochemistry [IHC] and enzyme-linked immunosorbent assay [ELISA]) on medial retropharyngeal lymph nodes and obex. More recent technologies centered on prion protein amplification methods of detection have shown promise as more sensitive and rapid CWD diagnostic tools. Here, we used blinded samples to test the efficacy of real time quaking-induced conversion (RT-QuIC) in comparison to ELISA and IHC for screening tissues, blood, and feces collected in 2019 from white-tailed deer in southeastern Minnesota, where CWD has been routinely detected since 2016. Our results support previous findings that RT-QuIC is a more sensitive tool for CWD detection than current antibody-based methods. Additionally, a CWD testing protocol that includes multiple lymphoid tissues (medial retropharyngeal lymph node, parotid lymph node, and palatine tonsil) per animal may effectively identify a greater number of CWD detections in a white-tailed deer population than a single sample type (i.e., medial retropharyngeal lymph nodes). These results reveal that the variability of CWD pathogenesis, sampling protocol, and testing platform must be considered for the effective detection and management of CWD throughout North America.

Key words: CWD, diagnostics, ELISA, IHC, RT-QuIC, sampling, testing protocol

INTRODUCTION

Chronic wasting disease (CWD) is a contagious, 100% fatal neurodegenerative disease affecting deer (*Odocoileus* spp.), moose (*Alces alces*), elk (*Cervus canadensis*), and reindeer (*Rangifer tarandus*). Classified as a transmissible spongiform encephalopathy (TSE), CWD is caused by a misfolded prion protein (PrP^{CWD}) which is shed through bodily fluids and can remain infectious in the environment for years (Williams ES, 1980; Prusiner, 1982). Originally detected in Colorado mule deer in 1967, CWD has been detected in additional cervid species and expanded in geographic distribution (Williams and Miller, 2002). As of February 2021, CWD has been found in at least 26 states and three Canadian provinces. The continued expansion of CWD across North America, and recent detections in Scandinavian countries (Mysterud *et al.*, 2020) is changing how cervids are hunted, managed, and consumed. For these reasons, stakeholders tasked with managing CWD must have access to the best diagnostic tools and relevant protocols.

The Minnesota Department of Natural Resources (MNDNR) has surveyed free-ranging white-tailed deer (*Odocoileus virginianus*) for CWD since detection in 2002 in farmed elk and free-ranging white-tailed deer in Minnesota and Wisconsin, respectively. Since then, CWD has been detected in 11 captive cervid facilities and 110 deer in Minnesota (> 90,000 tested), with the disease potentially established endemically in the southeast region, albeit at a low prevalence (La Sharr *et al.*, 2019). Surveillance efforts primarily utilize samples from hunter-harvested animals along with those collected opportunistically from deer found dead in poor body condition, euthanized deer displaying clinical signs of CWD, vehicle collisions, and targeted agency culling.

CWD diagnostic tests can be classified into two categories, first-generation antibody-based diagnostics (e.g., immunohistochemistry [IHC] and enzyme-linked immunosorbent assay [ELISA]) and second-generation prion protein amplification assays (e.g., real time quaking-induced conversion [RT-QuIC] and protein misfolding cyclic amplification [PMCA]). Management agencies have employed ELISA screening of medial retropharyngeal lymph nodes with confirmatory IHC on samples considered suspect by ELISA, as these have traditionally been considered the "gold standard" for CWD (Haley and Richt, 2017). ELISA and IHC, the currently available validated assays for CWD, must be completed at National Animal Health Laboratory Network laboratories. This diagnostic bottleneck has resulted in laboratories being at or beyond testing capacity (Schuler K, Abbott R, Mawdsley J, McGarvey K, January/February 2021). However, advances in CWD diagnostics allow for the implementation of new diagnostic standards and sampling techniques, a critical development as testing pressures and expectations increase (Haley and Richt, 2017; McNulty *et al.*, 2019; Bloodgood *et al.*, 2020; Henderson *et al.*, 2020). Amplification assays have been refined over the past decade and show potential as the next "gold standard" choice of diagnostic tools with increased sensitivity in a high-throughput platform (Haley and Richt, 2017).

We set out to examine the CWD detection capabilities of RT-QuIC test in comparison to ELISA and IHC tests using a population-level sample set of free-ranging deer from southeastern Minnesota. Few studies have investigated the utility of prion amplification assays in comparison to current immunodetection assays on free-ranging cervids (Haley *et al.*, 2014). Importantly, our experimental design included an assessment of RT-QuIC prion detection across multiple tissue types, blood, and feces collected from a subset of sampled

individuals. Insights gleaned from this approach will help inform future CWD surveillance efforts and cervid management across North America.

MATERIALS AND METHODS

Study area and experimental design

MNDNR contracted with United States Department of Agriculture-Wildlife Services to conduct culling within areas of known CWD-positive deer detections near Preston and Winona, Minnesota; a karst topography region with mixed upland hardwoods, swamp, and agricultural lands (22 January to 29 March 2019; Figure 1). Priority areas were designated spatially by Public Land Survey System sections (1 mi^2) with a high number of total CWD positive deer, positive female deer (considered to be disease anchors), or areas with high deer densities in close proximity to known positives. Whole, intact carcasses were transported to the Preston DNR Forestry office where MNDNR staff collected tissue samples - medial retropharyngeal lymph nodes (RPLN), submandibular lymph nodes (SLN), parotid lymph nodes (PLN), palatine tonsils (PT), feces, whole blood, and neck muscles (not included in this study). In some cases, significant coagulation of blood or lack of feces precluded collection. All samples were preserved at -20°C in the field.

In accordance with surveillance, RPLN samples from all individuals were tested by CWD ELISA at Colorado State University Veterinary Diagnostic Laboratory (CSU VDL) utilizing the Bio-Rad TeSeE Short Assay Protocol (SAP) Combo Kit (BioRad Laboratories Inc., Hercules, CA, USA). For each animal, a pooled homogenate was produced using 3 subsamples from each RPLN. Any suspect-positive RPLN from ELISA was confirmed through IHC of the prion protein as previously described (Hoover *et al.*, 2016).

RT-QuIC

Following transport from the field, bilateral PLN, SLN, and PT, blood, and feces were preserved at -80°C until RT-QuIC analysis was performed. All RT-QuIC research staff were blinded to the RPLN ELISA/IHC results.

Animal/sample identification: RT-QuIC screening began with unilateral sampling and testing of all 519 PLN. Samples that exhibited amyloid seeding activity were tested a second time to confirm the laboratory procedures and both results were reported to the MNDNR. MNDNR staff created a subset for further testing that included all deer that were CWD positive by ELISA/IHC on RPLN, exhibited amyloid seeding activity by RT-QuIC on unilateral PLN, and a randomly chosen set of RPLN ELISA/IHC not detected deer to reach an ELISA/IHC-blinded dataset of 60 animals.

In light of Bloodgood *et al.* (2020), lymphoid tissue sample preparation included bilateral sampling as described below. RT-QuIC testing was performed on bilateral PLN, bilateral SLN, and bilateral PT, as well as the available whole blood and fecal samples for the 60 animal subset. Additionally, the 13 ELISA/IHC CWD positive RPLN were provided by CSU VDL and tested by RT-QuIC. After samples were un-blinded, bilateral PLN, SLN, and PT subsamples of all deer that exhibited amyloid seeding activity on at least one tissue type by RT-QuIC were provided to CSU VDL for ELISA testing. CSU VDL staff were blinded to the RT-QuIC results.

Substrate Preparation: Recombinant hamster PrP (HaPrP90-231; provided by NIH Rocky Mountain Laboratory) was cloned into the pET41a(+) expression vector and was expressed in Rosetta™ (DE3) *E. coli* cells (Millipore Sigma, Darmstadt, Germany). Expression and purification of the recombinant substrate was performed following a modified version of the protocol from Orrù *et al.* (2017). Specifically, protein expression was induced using 0.75mM

IPTG in place of Overnight Express™ autoinduction (Novagen, Darmstadt, Germany), and cells were cracked with two passes at 16,000 psi on a microfluidizer rather than using a homogenizer.

Lymph Tissue Preparation: Lymph tissue (RPLN, PLN, SLN, and PT) dissections were initiated by a cross-sectional cut with sample collection along the cut face to reduce potential cross-contamination that may have originated during field collection. We utilized disposable forceps and scalpels and surface decontamination between samples (1:1.5; 5.25% sodium hypochlorite). Samples were dissected on fresh, disposable benchtop paper. A 10% (w:v) suspension was made by adding 100 mg of tissue to 900 uL of PBS. In the case of bilateral samples, 50 mg of each tissue was dissected and added to one tube. Tissue suspensions were then homogenized using 1.5 mm diameter zirconium oxide beads (Millipore Sigma, Burlington, Massachusetts, USA) and a BeadBug homogenizer (Benchmark Scientific, Sayreville New Jersey, USA), maximum speed for 90 seconds. Homogenized samples were stored at -80°C. Samples were diluted further to 10^{-3} in dilution buffer (0.1% SDS, 1X PBS, N2 supplement), and 2 µL were added to 98 µL of RT-QuIC master mix. The final tissue dilution factor in the reaction was 1:50,000.

Blood Preparation: We modified a protocol for blood preparation from Elder *et al.* (2015) and the phosphotungstic acid precipitation was first described by Safar *et al.* (1998). One mL of EDTA whole blood was placed in a tube with 1.5 mm diameter zirconium oxide beads and underwent four cycles of flash freeze-thaw consisting of 3 minutes in dry ice and 3 minutes at 37°C. It was then homogenized using a BeadBug homogenizer at top speed for 2 cycles (a total of 180s). The homogenate was centrifuged at 2,000 rpm for 2 minutes. We incubated 100 µl of supernatant with 7 µl of 4% (w/v) phosphotungstic acid (Sigma-Aldrich, St. Louis, Missouri, USA) in 0.2 M magnesium chloride. The product was then incubated in a ThermoMixer

(Eppendorf, Enfield, Connecticut, USA) at 37°C for 1h (1500 rpm) and subsequently centrifuged for 30 minutes at 14,800 rpm. The pellet was resuspended in 20 µL of dilution buffer. We added 2 µl of the 10^{-2}–diluted suspension to the RT-QuIC reaction described below.

Feces Preparation: We modified a fecal preparation protocol developed by Tennant *et al.* (2020). The fecal pellet was manually homogenized into 10% homogenates using 1X PBS. The solution was centrifuged at 3,000 rpm for 15 minutes at 4°C. We then centrifuged 500 µl of supernatant at 15,000 rpm for 30 minutes. The pellet was resuspended in 100 µl of 1X PBS subsequently incubated with 7 µl of phosphotungstic acid solution as described above. The solution was then centrifuged for 30 minutes at 14,800 rpm. The pellet was resuspended in 10 µl 0.1% SDS in PBS. We added 2 µl of the suspension to the RT-QuIC reaction described below.

Assay parameters: Recombinant hamster PrP substrate was filtered at 3,000 x g through a 100 kDa molecular weight cutoff spin column. A master mix was made to the following concentrations: 1X PBS, 500 uM EDTA, 50 uM Thioflavin T, 300 mM NaCl, and 0.1 mg/mL rPrP. All reagents were filter-sterilized through 0.22um PVDF filters. We pipetted 98 uL of the master mix into each well on a black 96-well plate with clear bottoms. After samples were added, the plate was sealed with clear tape. Plates were shaken on a BMG FLUOstar® Omega microplate reader (BMG LABTECH Inc., Cary, North Carolina, USA) at 700 rpm, double orbital for 57 sec and then rested for 83 sec. This shake/rest cycle repeated 21 times, then the fluorescence was recorded. For all tissue types, the temperature was set to 42°C. The whole shake/read cycle would be repeated 58 times for a total of ~46 hr. Readings were recorded with an excitation filter of 450 nm and an emission filter of 480 nm. The gain was set to 1600. The machine performed 21 flashes/well.

RT-QuIC Data Analysis: Rate of amyloid formation was determined as the inverse of hours (1/h) for amyloid seeding activity to surpass a threshold of 10 standard deviations above the average baseline readings after 4.5 hr for each plate (Hoover *et al.*, 2016). A minimum of four replicates were performed for each sample. Samples were considered putative positive when at least 50% of the replicates gave a fluorescence signal higher than the threshold cut-off value. P-values were calculated based on rate of amyloid formation versus negative controls using a Mann Whitney U-test as described by Tennant *et al.* (2020) to determine the statistically significant difference in rate of amyloid formation between samples tested and negative controls on the respective plates. Statistical significance was established at 0.05 ($\alpha = 0.05$) and p-values below 0.05 were considered statistically different. We additionally examined statistical significance for all RT-QuIC data using a maxpoint ratio analysis (Vendramelli *et al.*, 2018; Supplemental File 1). All statistical analyses were performed using GraphPad Prism software (version 9). We report 95% Wilson Score confidence limits for proportions, which is appropriate for small sample size and sample proportions close to 0 or 1 (Brown, Cai and DasGupta, 2001). Confidence limits were estimated using Epitools Epidemiological Calculators (Ausvet, Bruce, ACT, Australia).

RESULTS

First generation surveillance: ELISA and IHC

Demographics of the 519 culled deer were as follows: 205 (39%) adult female, 66 (13%) adult male, 36 (7%) yearling female, 53 (10%) yearling male, 82 (16%) fawn female, and 77 (15%) fawn male.

Of the 519 deer, RPLN from 13 deer (0.025; 95% Wilson confidence limits [95% CL] = 0.015 – 0.042) were reported as CWD positive by CSU VDL (Figure 1; Table 1). Demographics

of the 13 CWD ELISA/IHC positive deer were as follows: 9 (69%) adult female, 3 (23%) adult male, 0 yearling female, 1 (8%) yearling male, 0 fawn female, and 0 fawn male.

Second generation surveillance: RT-QuIC

The first objective for RT-QuIC analysis was a blinded screening of the 519 deer using unilateral PLN samples. The first analysis identified 11 (0.021; 95% CL = 0.012 – 0.038) samples exhibiting significant ($p<0.05$) amyloid seeding activity with repeated results upon retesting (Table 1). We next examined the diagnostic agreement between these 11 animals, animals previously classified as CWD-positive by ELISA/IHC (n=13), and a series of ELISA negative controls (total n=60). One animal that was positive by ELISA/IHC (MN100528) was not included in the 60-deer sample set, but had the same tissue set screened subsequently (following unblinding). Demographics of the 61-deer sample set were as follows: 32 (52%) adult female, 10 (16%) adult male, 5 (8%) yearling female, 4 (7%) yearling male, 6 (10%) fawn female, and 4 (7%) fawn male.

RT-QuIC results indicated 16 (26%) of 61 deer exhibited significant ($p<0.05$) amyloid seeding activity in at least one sample type. By sample type, we observed amyloid seeding activity in: 12/61 (0.20; 95% CL = 0.12 - 0.31) bilateral PLN, 12/61 (0.20; 95% CL = 0.12 – 0.31) bilateral SLN, 13/61 (0.21; 95% CL = 0.13 – 0.33) bilateral PT, 13/13 (1.0; 95% CL = 0.77 – 1.0) bilateral RPLN, 7/51 (0.14; 95% CL = 0.07 – 0.26) whole blood, and 5/47 (0.11; 95% CL = 0.05 – 0.23) feces (Table 1; Figure 2). There was intra-individual variability in the types of tissues that exhibited amyloid seeding activity for each of the 16 animals (Figures 2,3). We detected amyloid seeding activity beneath levels of significance ($p<0.05$) in lymphoid and blood samples (n=6) from several of these 16 animals, and considered them putative positive samples (Table 1; Figure 2). Demographics of the 16 deer were as follows: 9 (56%) adult female, 4 (25%)

adult male, 0 yearling female, 2 (13%) yearling male, 0 fawn female, and 1 (6%) fawn male. Additionally, amyloid seeding was detected in 2 of 4 replicates (putative positive; not significant) within a palatine tonsil sample from a single animal (MN145287; adult, female) that had no indication of amyloid seeding activity in any other sample type and was ELISA negative across all sample types (Table 1; Figure 2). No other samples from the 61 deer demonstrated amyloid seeding activity.

ELISA analysis of animals exhibiting amyloid seeding activity by RT-QuIC

To provide tissue-specific comparison between ELISA and RT-QuIC, PLN, SLN, and PT samples (n=51) from the 17 RT-QuIC positive animals were blind tested by ELISA at CSU VDL. Fifty of the 51 samples demonstrated RT-QuIC and ELISA results agreement (Table 1). MN145273 PT exhibited significant ($p<0.05$) amyloid seeding activity by RT-QuIC and was not detected by ELISA. Additionally, four samples (MN100528 SLN, MN137219 PT, MN137346 PLN, MN145287 PT) demonstrated amyloid seeding activity that was beneath levels of significance ($p<0.05$) and were not detected by ELISA. Samples positive on both testing platforms showed a moderate positive correlation of the semi-quantitative measures of prion content ($R^2=0.581$; Figure 4). Samples with an ELISA OD value of 3.999 did not bias the correlation as a similar R^2 was derived from the sample set with those samples removed (data not shown).

DISCUSSION

The goal for diagnostic assays is to optimize the detection of the target so that animals that are diseased or infected are identified as positive (sensitivity) and animals that are not are identified as negative (specificity). An array of factors affect this optimization including, but not limited to, sample type and integrity, sampling method, assay elements, and individual animal

(e.g. physiologic or immune state, genetics) and population characteristics (e.g. pathogen prevalence). For CWD, disease detection has primarily been based on ELISA and IHC and the use of postmortem medial retropharyngeal lymph node and obex samples. This approach is effective at identifying animals with end-stage disease, but is limited in detecting low levels of prion burden seen in early stages of disease (Haley and Richt, 2017; Hoover *et al.*, 2017; McNulty *et al.*, 2019). We evaluated the performance of RT-QuIC in comparison to these immunodetection assays with results that support RT-QuIC's greater potential to detect low levels of CWD prion by utilizing a diverse tissue set that may have value for early detection of the disease.

A growing body of research collectively provides strong evidence that second-generation amplification-based assays have much greater sensitivity in the detection of misfolded PrP than first-generation tests (Haley and Richt, 2017, Soto and Pritzkow, 2018, McNulty *et al.*, 2019). Our results support this conclusion as varied tissue types exhibited amyloid seeding activity with RT-QuIC, but ELISA testing resulted in no detection of PrP^{CWD} in the specific tissue type or within the animal. This led to the discovery of four putative CWD-positive animals by RT-QuIC out of a pool of 48 CWD ELISA not detected animals (RPLN), as well as two ELISA-positive animals (RPLN) with additional putative positive sample types. Collectively, these six animals demonstrated intra-animal variability in CWD positive tissues (ELISA and/or RT-QuIC). Based on relatively low ELISA OD values of the positive tissues in these six animals, and given that RT-QuIC identified tissues that exhibited amyloid seeding activity but were not detected by ELISA, we posit that these six animals were either in the early stages of CWD infection or represent RT-QuIC false-positives. We believe that the former is the case as zero amyloid seeding activity was observed in the 44 "negative" animals (i.e., zero of four replicates) and there

was no indication of inter-animal or intra-animal cross-contamination from the sample collection, preparation, or RT-QuIC processes based on the spatial and chronological distribution of CWD detections and the reliability of plate-level controls.

It is problematic to confirm the status of RT-QuIC-positive samples that are negative by first-generation methods as amplification assays approach attogram levels of misfolded prion protein detection, multifold beyond the limits of immunodetection methods (Haley, Richt, *et al.*, 2018). However, similar studies focused on longitudinal analyses of CWD infection support claims that amplification-based assays can effectively detect CWD infections prior to both ELISA and IHC (Haley, Henderson, *et al.*, 2018; Denkers *et al.*, 2020; Haley *et al.*, 2020; Henderson *et al.*, 2020). Previous studies also indicate that the unequal tissue distribution of CWD prion protein in early stages of the disease, coupled with sectioning or sampling technique variability and limitations of immunodetection assays, contributes to the apparent reduced sensitivity of ELISA and IHC (Hoover *et al.*, 2017; Bloodgood *et al.*, 2020). Another complicating factor when comparing first- and second-generation CWD assays, is the potential for variable strains of PrP^{CWD} to be detected by RT-QuIC or PMCA but missed by immunodetection methods due to strain sensitivity to enzymatic digestion. ELISA, IHC, and western-blotting methods utilize antibodies that are unable to distinguish between PrP and PrP^{CWD}, thus necessitating Proteinase-K digestion to identify PrP strains resistant to degradation. Recent data indicate that a variety of PrP^{CWD} strains are circulating in cervids, raising the possibility that diagnostic methods which do not utilize enzymatic digestion have greater potential for identifying a broader family of PrP^{CWD} (Duque Velásquez *et al.*, 2015; Osterholm *et al.*, 2019). At the same time, the potential for naturally occurring, non-infectious, conformations of healthy PrP to cause false-positives within amplification-based analyses must be considered.

Sample type also influences CWD test sensitivity, particularly early in infection. RT-QuIC has documented amyloid seeding activity in feces and blood of experimentally-infected deer in the preclinical stages and shortly after inoculation, respectively (M. Elder *et al.*, 2015; Tennant *et al.*, 2020). In both studies, optimal sample conditions - limiting freeze/thaw in feces and heparin preservation in blood - presented the most consistent results. However, these conditions were not reproduced here, which may have contributed to the variable RT-QuIC results from feces and blood. The pathogenesis of CWD provides valuable insight into the prion seeding activity we observed in PT. Tonsillar tissue is one of the first tissues to demonstrate prion immunodeposition, which fits with observed prion traffic through the lymphatic system and is supported in this study (Haley and Richt, 2017; Hoover *et al.*, 2017; Henderson *et al.*, 2020). Palatine tonsil tissue from multiple animals, possibly in early stages of the disease, exhibited amyloid seeding activity by RT-QuIC and had either low OD values or were not detected by ELISA. These results support the value of PT as a tissue of choice for CWD surveillance, particularly when utilizing RT-QuIC. Based on our results, PT demonstrated higher levels of amyloid seeding activity (i.e. “hotter” samples) than did RPLN, yet a majority of both sample types were at the upper limits of detection by ELISA. This leads to the possibility that while RPLN may be good for ELISA-based surveillance, PT may be the ideal tissue type for RT-QuIC-based surveillance. Although the feasibility of identifying PT versus RPLN in field extraction should be considered, further investigation is warranted. We also document the potential for RT-QuIC screening of PLN to identify early stages of CWD infection, and our results supported the continued importance of RPLN for CWD surveillance. Collectively, our RT-QuIC data provide evidence that multi-tissue sampling would provide the best sensitivity for discovering early-infected animals through post-mortem CWD surveillance - likely a

combination of PLN, RPLN, and PT, as well as properly collected feces and blood. Fiscal limitations must be accounted for when considering sample type and testing protocols and therefore pooled tissue sample testing may be most appropriate.

Detecting early-infected animals through CWD surveillance is imperative for controlling the disease. This is particularly true for geographic locations where CWD has not been detected. As demonstrated throughout the history of CWD, earlier disease discovery on the landscape leads to earlier implementation of management and more effective control (Miller and Fisher, 2016). The efficacy of tools and protocols for early detection is likely of limited use in core endemic areas where disease is well established, however, implementation of such approaches at the periphery of endemic areas and within areas of incipient CWD expansion events would be critical for detecting and removing newly infected animals, thus limiting the spread of CWD. We posit that the growing number of studies documenting the utility of RT-QuIC for the surveillance of a variety of protein-misfolding and prion diseases, including CWD, collectively demonstrate the method is robust and will greatly aid our understanding and control of CWD (Wilham *et al.*, 2010; Orrú *et al.*, 2015; Caughey *et al.*, 2017; Franceschini *et al.*, 2017; Cooper *et al.*, 2019; Saijo *et al.*, 2019; Henderson *et al.*, 2020; Rossi *et al.*, 2020). In light of the results reported herein, we recommend the implementation of advanced, second-generation, CWD diagnostic assays into existing CWD surveillance, management, and regulatory initiatives.

Future investigation, building on these discoveries, will include optimization of a sampling protocol for CWD amplification assays to increase detection sensitivity, elucidating potential impacts of *PRNP* allele variation on testing for particular CWD strains, and continued analyses of multi-tissue samples secured from CWD positive regions. These efforts, combined with robust statistical validation of new CWD diagnostic tests will help to establish a more

complete depiction of the CWD landscape. Our results, utilizing a blinded sample set of free-ranging white-tailed deer from documented CWD hotspots, indicate that RT-QuIC may be a more powerful tool than current methods in identifying CWD positive animals in coordination with a multi-tissue sample collection protocol, particularly for early infections and in CWD-free locations, whether free-ranging or farmed. These findings have direct implications for the effective surveillance and management of CWD across all cervids.

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LITERATURE CITED

Bloodgood, J. *et al.* (2020) 'Chronic Wasting Disease Diagnostic Discrepancies: The Importance of Testing Both Medial Retropharyngeal Lymph Nodes', *Journal of wildlife diseases*. doi: 10.7589/JWD-D-20-00007.

Brown, L. D., Cai, T. T. and DasGupta, A. (2001) 'Interval Estimation for a Binomial Proportion', *Statistical science: a review journal of the Institute of Mathematical Statistics*, 16(2), pp. 101–117.

Caughey, B. *et al.* (2017) 'Amplified Detection of Prions and Other Amyloids by RT-QuIC in Diagnostics and the Evaluation of Therapeutics and Disinfectants', *Progress in molecular biology and translational science*, 150, pp. 375–388.

Cooper, S. K. *et al.* (2019) 'Detection of CWD in cervids by RT-QuIC assay of third eyelids', *PloS one*, 14(8), p. e0221654.

Denkers, N. D. *et al.* (2020) 'Very low oral exposure to prions of brain or saliva origin can transmit chronic wasting disease', *PloS one*, 15(8), p. e0237410.

Duque Velásquez, C. *et al.* (2015) 'Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains', *Journal of virology*, 89(24), pp. 12362–12373.

Elder, A. M. *et al.* (2015) 'Immediate and Ongoing Detection of Prions in the Blood of Hamsters and Deer following Oral, Nasal, or Blood Inoculations', *Journal of virology*, 89(14), pp. 7421–7424.

Franceschini, A. *et al.* (2017) 'High diagnostic value of second generation CSF RT-QuIC across the wide spectrum of CJD prions', *Scientific reports*, 7(1), p. 10655.

Haley, N. J. *et al.* (2014) 'Detection of chronic wasting disease in the lymph nodes of free-ranging cervids by real-time quaking-induced conversion', *Journal of clinical microbiology*, 52(9), pp. 3237–3243.

Haley, N. J., Henderson, D. M., *et al.* (2018) 'Chronic wasting disease management in ranched elk using rectal biopsy testing', *Prion*, 12(2), pp. 93–108.

Haley, N. J., Richt, J. A., *et al.* (2018) 'Design, implementation, and interpretation of amplification studies for prion detection', *Prion*, 12(2), pp. 73–82.

Haley, N. J. *et al.* (2020) 'Cross-validation of the RT-QuIC assay for the antemortem detection of chronic wasting disease in elk', *Prion*, 14(1), pp. 47–55.

Haley, N. J. and Richt, J. A. (2017) 'Evolution of Diagnostic Tests for Chronic Wasting Disease, a Naturally Occurring Prion Disease of Cervids', *Pathogens*, 6(3). doi: 10.3390/pathogens6030035.

Henderson, D. M. *et al.* (2020) 'Progression of chronic wasting disease in white-tailed deer analyzed by serial biopsy RT-QuIC and immunohistochemistry', *PloS one*, 15(2), p. e0228327.

Hoover, C. E. *et al.* (2016) 'Detection and quantification of CWD prions in fixed paraffin embedded tissues by real-time quaking-induced conversion', *Scientific reports*, 6, p. 25098.

Hoover, C. E. *et al.* (2017) 'Pathways of Prion Spread during Early Chronic Wasting Disease in Deer', *Journal of virology*, 91(10). doi: 10.1128/JVI.00077-17.

La Sharr, K. *et al.* (2019) *Surveillance and management of chronic wasting disease in Minnesota*. Minnesota Department of Natural Resources.

McNulty, E. *et al.* (2019) 'Comparison of conventional, amplification and bio-assay detection methods for a chronic wasting disease inoculum pool', *PloS one*, 14(5), p. e0216621.

M. Elder, A. *et al.* (2015) 'Longitudinal analysis of blood-borne prion infection', *European Journal of Molecular & Clinical Medicine*, 2(4-5), p. 128.

Miller, M. W. and Fisher, J. R. (2016) 'The First Five (or More) Decades of Chronic Wasting Disease: Lessons for the Five Decades to Come', in *Transactions of the 81st North American Wildlife and Natural Resources Conference; Special Session Three: Science-Based Management Strategies for Fish and Wildlife Diseases*. *81st North American Wildlife and Natural Resources Conference*, Wildlife Management Institute, pp. 110–120.

Mysterud, A. *et al.* (2020) 'Policy implications of an expanded chronic wasting disease universe', *The Journal of applied ecology*, (1365-2664.13783). doi: 10.1111/1365-2664.13783.

Orrú, C. D. *et al.* (2015) 'Rapid and sensitive RT-QuIC detection of human Creutzfeldt-Jakob disease using cerebrospinal fluid', *mBio*, 6(1). doi: 10.1128/mBio.02451-14.

Orrù, C. D. *et al.* (2017) 'RT-QuIC Assays for Prion Disease Detection and Diagnostics', *Methods in molecular biology* , 1658, pp. 185–203.

Osterholm, M. T. *et al.* (2019) 'Chronic Wasting Disease in Cervids: Implications for Prion Transmission to Humans and Other Animal Species', *mBio*, 10(4). doi: 10.1128/mBio.01091-19.

Prusiner, S. B. (1982) 'Novel proteinaceous infectious particles cause scrapie', *Science*, 216(4542), pp. 136–144.

Rossi, M. *et al.* (2020) 'Ultrasensitive RT-QuIC assay with high sensitivity and specificity for Lewy body-associated synucleinopathies', *Acta neuropathologica*, 140(1), pp. 49–62.

Safar, J. *et al.* (1998) 'Eight prion strains have PrPSc molecules with different conformations', *Nature Medicine*, 4(10), pp. 1157–1165.

Saijo, E. *et al.* (2019) 'Ultrasensitive RT-QuIC Seed Amplification Assays for Disease-Associated Tau, α-Synuclein, and Prion Aggregates', *Methods in molecular biology* , 1873, pp. 19–37.

Schuler K, Abbott R, Mawdsley J, McGarvey K (January/February 2021) 'Getting results in the fight against CWD', *The Wildlife Professional*. Edited by E. Thompson, 15(1), pp. 36–39.

Soto, C. and Pritzkow, S. (2018) 'Protein misfolding, aggregation, and conformational strains in neurodegenerative diseases', *Nature neuroscience*, 21(10), pp. 1332–1340.

Tennant, J. M. *et al.* (2020) 'Shedding and stability of CWD prion seeding activity in cervid feces', *PloS one*, 15(3), p. e0227094.

Vendramelli, R. *et al.* (2018) 'ThermoMixer-Aided Endpoint Quaking-Induced Conversion (EP-QuIC) Permits Faster Sporadic Creutzfeldt-Jakob Disease (sCJD) Identification than Real-Time Quaking-Induced Conversion (RT-QuIC)', *Journal of clinical microbiology*, 56(7). doi: 10.1128/JCM.00423-18.

Wilham, J. M. *et al.* (2010) 'Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays', *PLoS pathogens*, 6(12), p. e1001217.

Williams, E. S. and Miller, M. W. (2002) 'Chronic wasting disease in deer and elk in North America', *Revue scientifique et technique* , 21(2), pp. 305–316.

Williams ES, Y. S. (1980) 'Chronic wasting disease of captive mule deer: A spongiform encephalopathy', *Journal of wildlife diseases*, 16(1), pp. 89–98.

Table 1 - RT-QuIC and ELISA results on animals from the 60 white-tailed deer (*Odocoileus virginianus*) subset that indicated ELISA positivity and/or exhibited amyloid seeding activity by RT-QuIC on at least one sample type.[a] X=statistically significant amyloid seeding activity determined by Mann-Whitney test and Dunnet's test ($p<0.05$; see Supplemental Methods) O=none or not statistically significant amyloid seeding activity determined by Mann-Whitney test and Dunnet's test ($p<0.05$; see Supplemental Methods)

		Unilateral parotid LN		Bilateral medial retropharyngeal LN		Bilateral parotid LN		Bilateral submandibular LN		Bilateral palatine tonsil			
DNR ID	Sex, age	RT-QuIC 1st run	RT-QuIC 2nd run	RT-QuIC	ELISA	RT-QuIC	ELISA	RT-QuIC	ELISA	RT-QuIC	ELISA	Blood	Feces[d]
MN137315	Female, adult	X	X	X	3.999	X	3.263	X	3.999	X	3.999	O[b]	O
MN137450	Male, yearling	X	X	X	3.999	X	3.999	X	3.999	X	3.999	X	O
MN106838	Male, adult	X	X	X	3.999	X	0.431	X	2.006	X	3.999	—	—
MN145282	Female, adult	X	X	X	3.999	X	3.999	X	3.999	X	3.999	X	X
MN137289	Female, adult	X	X	X	3.999	X	3.390	X	3.999	X	3.999	X	X
MN137471	Female, adult	X	X	X	3.999	X	0.167	X	3.999	X	3.999	X	X
MN137479	Female, adult	X	X	X	3.999	X	0.310	X	3.999	X	3.999	X	O
MN137193	Male, adult	X	X	X	3.999	X	3.999	X	3.035	X	3.999	O[c]	O
MN101141	Female, adult	X	X	X	3.999	X	0.320	X	3.999	X	3.999	O[c]	—
MN145279	Female, adult	X	X	X	3.999	X	3.999	X	3.999	X	3.999	X	O
MN137293	Female, adult	X	X	X	3.287	X	3.189	X	3.999	X	3.999	X	X
MN100528	Female, adult	O	O	X	3.188	O	ND	O[b]	ND	X	0.468	O	—
MN137219	Male, adult	O	O	X	1.315	O	ND	O	ND	O[b]	ND	O	O
MN137346	Male, fawn	O	O	—	ND	O[b]	ND	O	ND	O	ND	—	X
MN137203	Male, yearling	O	O	—	ND	X	0.311	O	ND	O	ND	O[b]	O
MN145273	Male, adult	O	O	—	ND	O	ND	O	ND	X	ND	O	O
MN145287	Female, adult	O	O	—	ND	O	ND	O	ND	O[b]	ND	O	O

[a] — = data not available because samples were not available.
[b] Results demonstrate at least 50% of wells exhibited amyloid seeding activity by RT-QuIC but were not statistically significant by Mann-Whitney test and Dunnet's test (putative positive; $p<0.05$; see Supplemental Methods).
[c] Blood sample coagulated. Non-coagulated blood should be used for RT-QuIC prion detection (Elder *et al.*, 2015).
[d] Best practices for RT-QuIC on fecal samples indicate fresh collection (at time of defication or death) and flash freezing for optimal prion amplification detection. These conditions were not reproduced in this study (Tennant *et al.*, 2020).

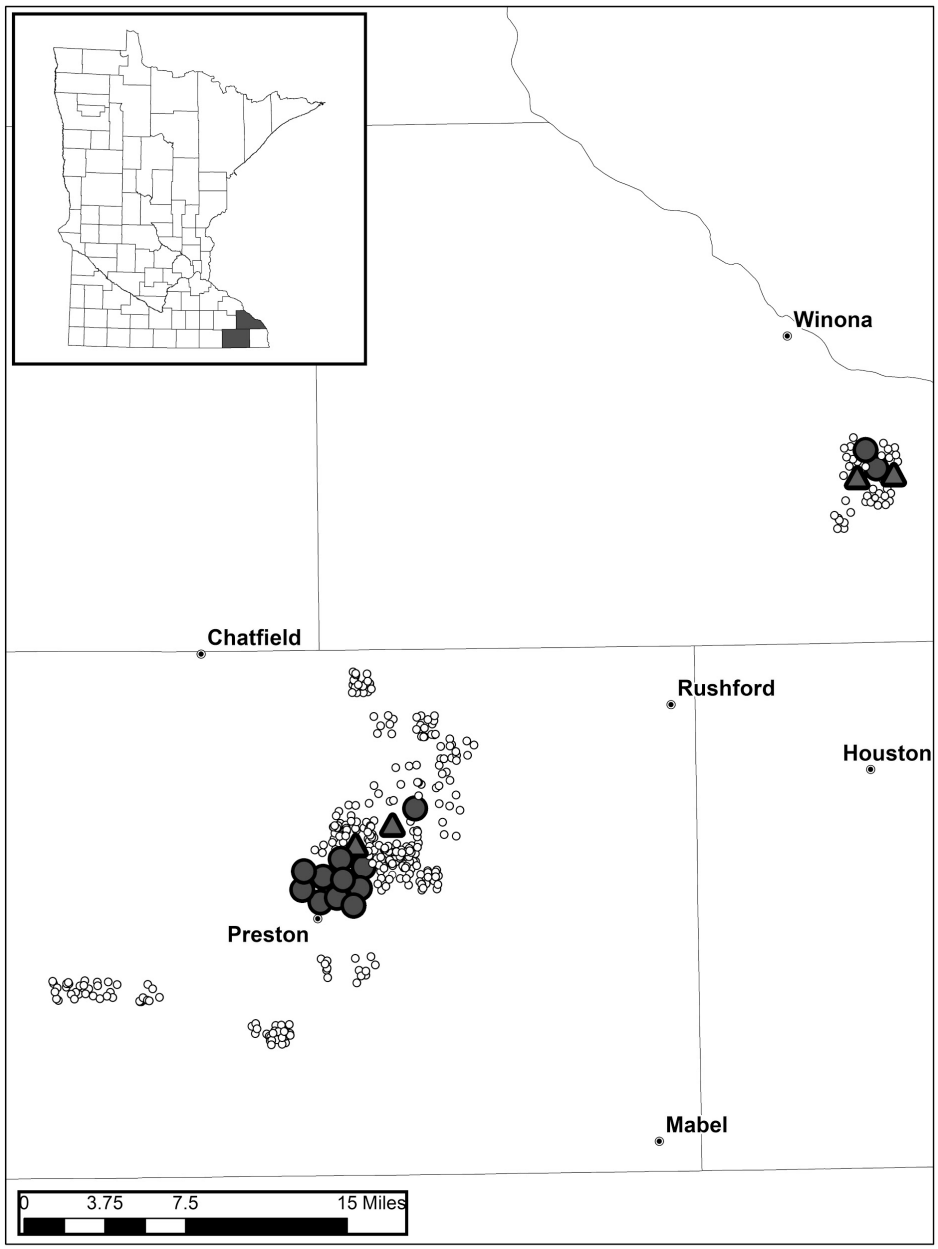

Figure 1 - Locations of the 519 white-tailed deer (*Odocoileus virginianus*) collected during the 2019 Minnesota Department of Natural Resources agency culling. Thirteen deer identified as CWD ELISA, IHC, and RT-QuIC (see Results) positive by medial retropharyngeal lymph node samples are indicated by large circles. Four additional deer identified as CWD putative positives by RT-QuIC are indicated by large triangles.

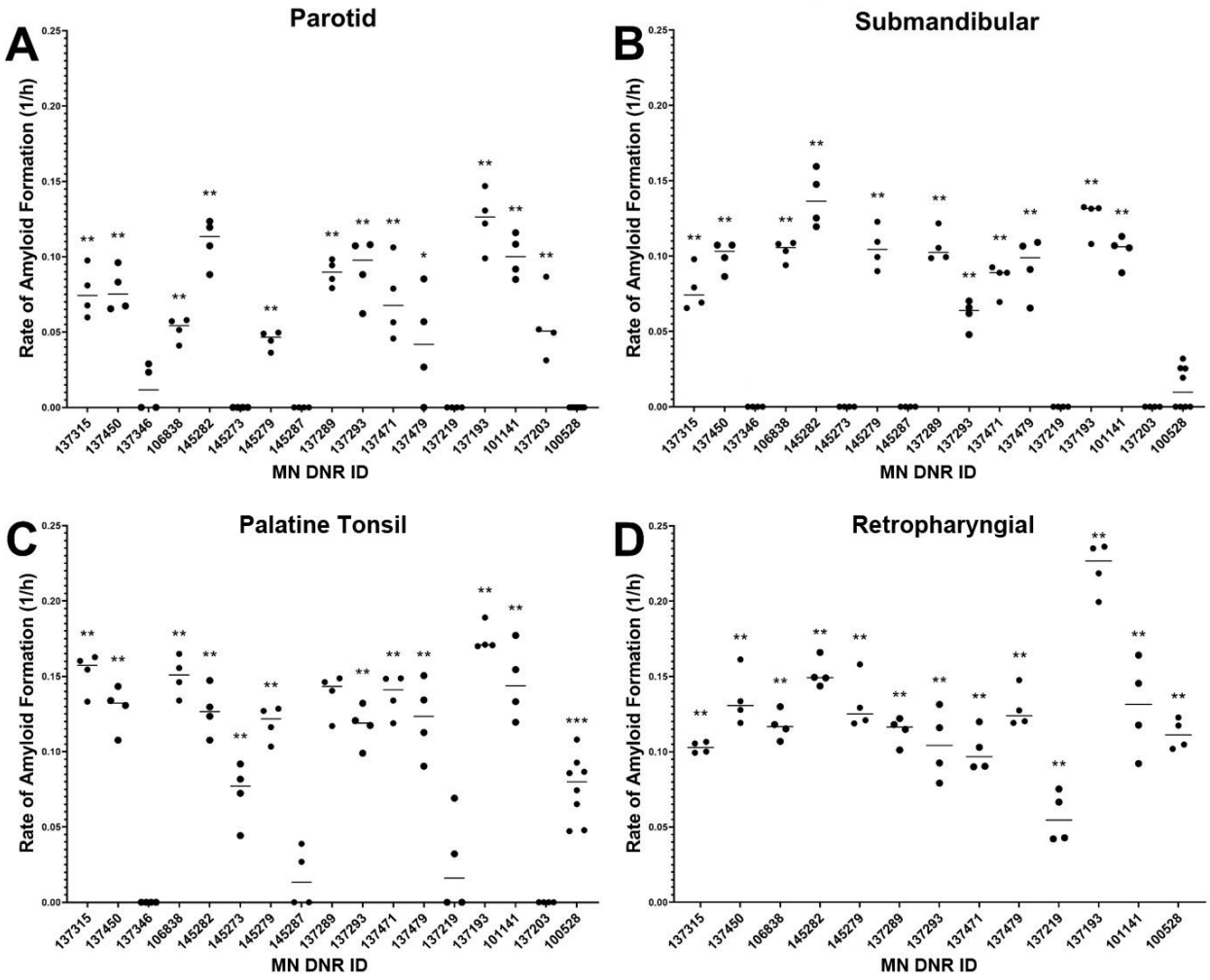

Figure 2 - Relative rates of amyloid formation (1/h) of lymphoid tissue samples from the 60 white-tailed deer (*Odocoileus virginianus*) subset that exhibited amyloid seeding activity by RT-QuIC. Samples exhibiting amyloid seeding activity were deemed positive by Mann-Whitney test and Dunnet's test (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; see Supplemental Methods).

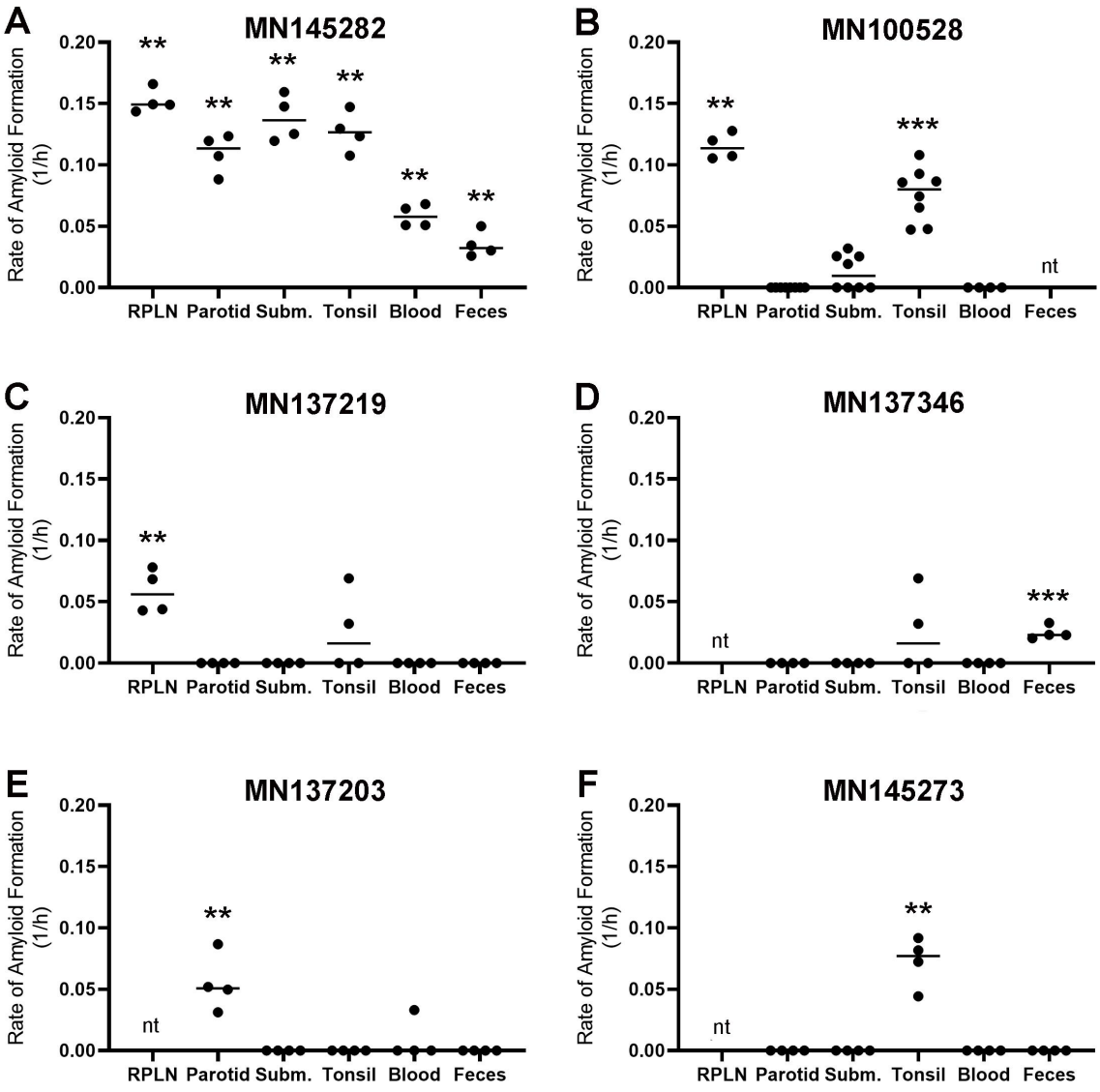

Figure 3 - Relative rates of amyloid formation (1/h) of all sample types from six white-tailed deer (*Odocoileus virginianus*) that exhibited amyloid seeding activity by RT-QuIC. Note the variability in amyloid seeding activity across animals. "**A**" demonstrates a highly CWD positive animal across all sample types. "**B**" is likely an animal in earlier disease progression when compared to "**A**". "**C - F**" are likely animals with very early infection in that only one sample type is statistically significant. Note that "**D - F**" did not have medial retropharyngeal lymph

nodes (RPLN) available to test as they were not detected by ELISA and thus discarded at CSU VDL prior to us having access. Samples exhibiting amyloid seeding activity were deemed positive by Mann-Whitney test and Dunnet's test (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; see Supplemental Methods). Those samples that were not available for RT-QuIC testing are marked with "nt" (not tested).

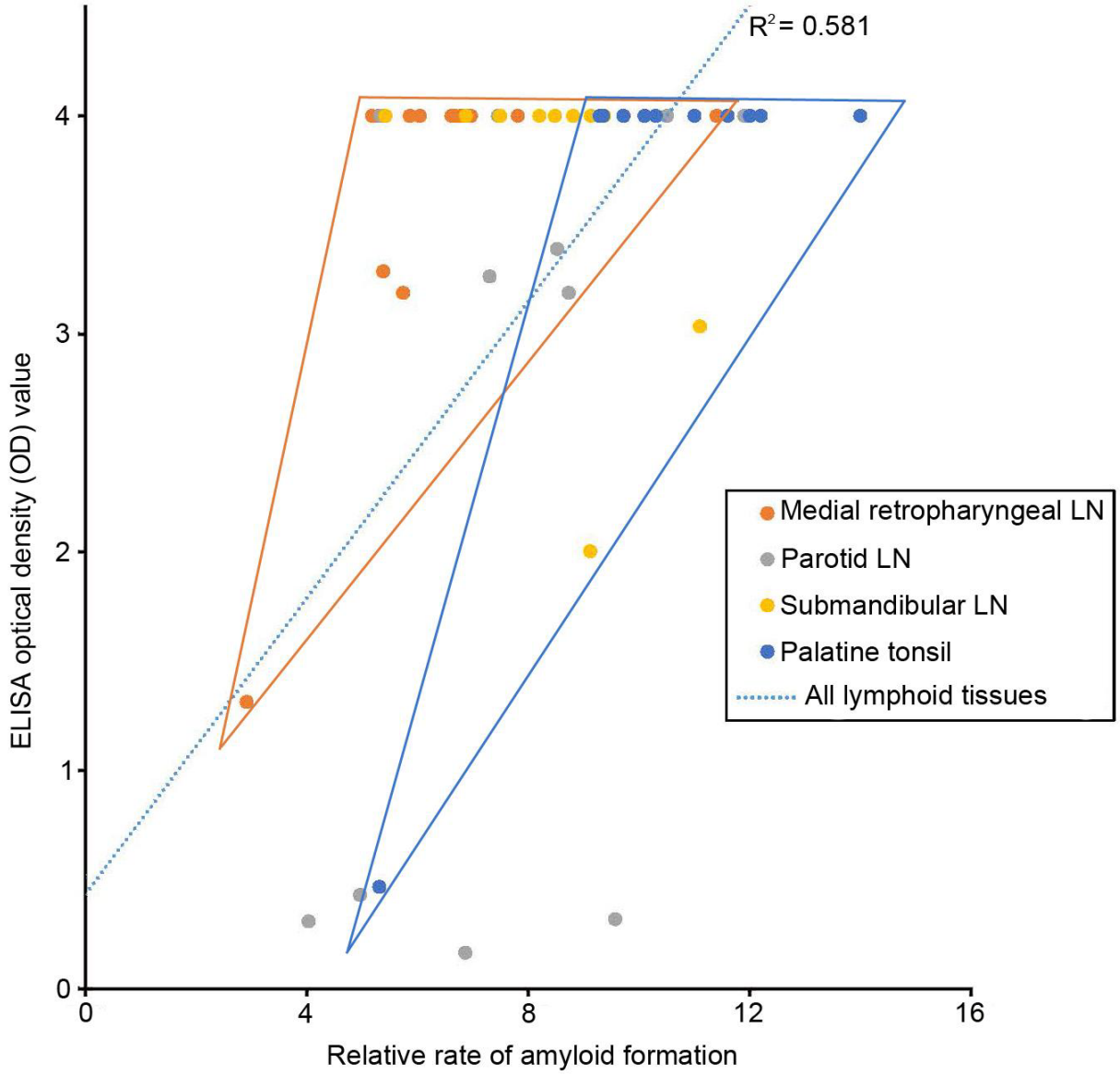

Figure 4 - Relationship between the relative rate of amyloid formation and ELISA optical density (OD) for lymphoid tissues examined herein. Depicted samples revealed both amyloid seeding activity by RT-QuIC and had OD values between 0.100 and 3.999. A moderate positive correlation is appreciated across all lymphoid tissue samples (dotted line; Pearson's correlation coefficient, $R^2 = 0.581$). The relationship between medial retropharyngeal lymph node (RPLN) and palatine tonsil (PT) samples is appreciated in that RPLN samples had a generally lower rate of amyloid formation than PT samples.

Chronic Wasting Disease: MNPRO Research Update

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19 April 2021

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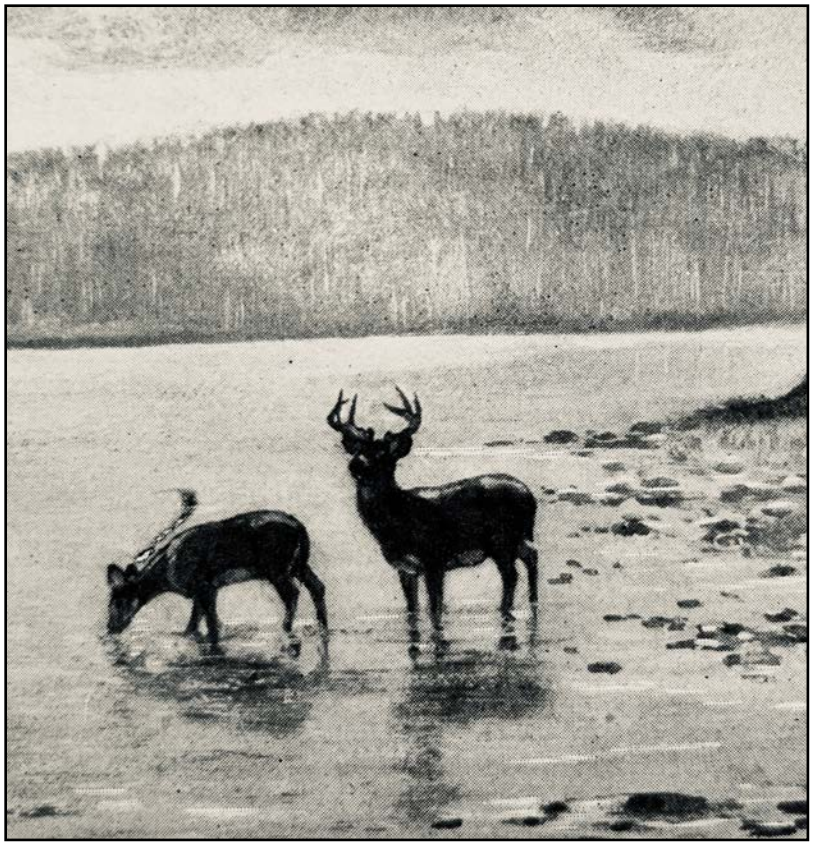
Attach. G

Background: Chronic Wasting Disease

- Direct threat to Minnesota's white-tailed deer, elk, and moose
- CWD positive deer can pass infectious prions to other deer and shed them into the environment
- Poses a risk to multiple economic sectors in the state and across the USA
- Requires an immediate and sustained research and outreach effort
- Must do all that we can to protect the rich heritage surrounding cervids



Background: Minnesota Center for Prion Research and Outreach (MNPRO)

- Began as a grassroots effort in 2019 to assist the state and nation with CWD research and outreach. Co-Director **Dr. Tiffany Wolf**, Associate Director and Program Manager **Marc Schwabenlander**

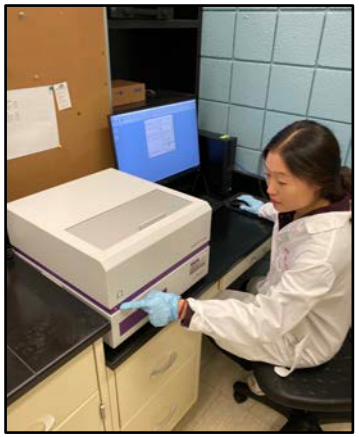
- 2019: MNPRO research team awarded ~$2M by the state (MN Legislature: LCCMR; Minnesota Extension Rapid Ag Response Fund) to develop advanced CWD diagnostic tools

- Immediate need for faster and more sensitive diagnostics with live-animal, harvested animal, and environmental application

Tiffany Wolf, DVM, PhD

Marc Schwabenlander, MPH

CWD Diagnostic Test Research

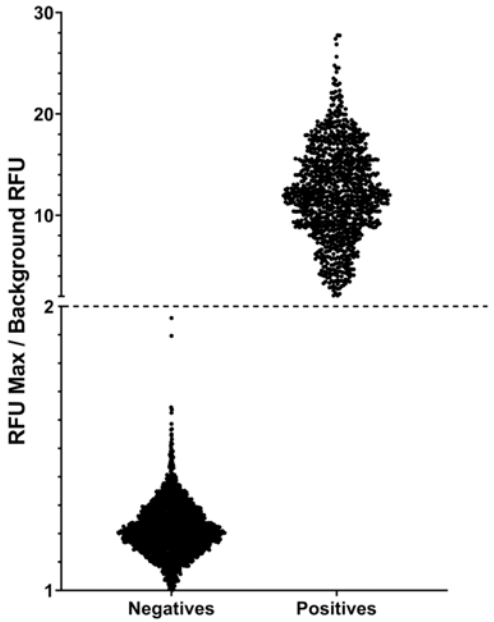


- Major milestone reached on 24 Jan 2020
- RT-QuIC functionality:
  - Highly sensitive and robust prion detection assay
    - ”Can detect equivalent of ~1 tablespoon of CWD prions in ~400 Olympic-sized swimming pools of water”
  - **Live animal test, harvested animal test, and environmental test**
  - MNPRO lab only lab in state with RT-QuIC
    - Protocols for tissues, blood, feces, water, plants
    - Soil protocol in development (online summer 2021)
  - Assisting USDA with official validation using tissues (lymph nodes, brainstem, rectal biopsy)
  - Working to establish nation-wide RT-QuIC network

RT-QuIC

- Collaboration with DNR to internally validate RT-QuIC
  - Blinded analysis of ~500 deer. RT-QuIC was 100% accurate using same tissues used for existing diagnostics (ELISA, IHC). Schwabenlander et al. (*In review*)
    - Multiple tissue types (i.e., tonsils, and several lymph nodes) might ID more CWD+ animals
  - **Still in research phase with DNR**
    - **Additional staff, equipment, lab space required to expand testing capacity. Epidemiological validation in progress (Dr. Tiffany Wolf)**
- Helping to establish self-sustaining Tribal CWD network in MN/WI/MI
  - 2020-2021: Worked with 5 MN tribes + 1854 Treaty Authority to secure tissues (including from Dakota Co. and Beltrami Co.)
- BAH and USDA: environmental samples and tissues from depopulated herds

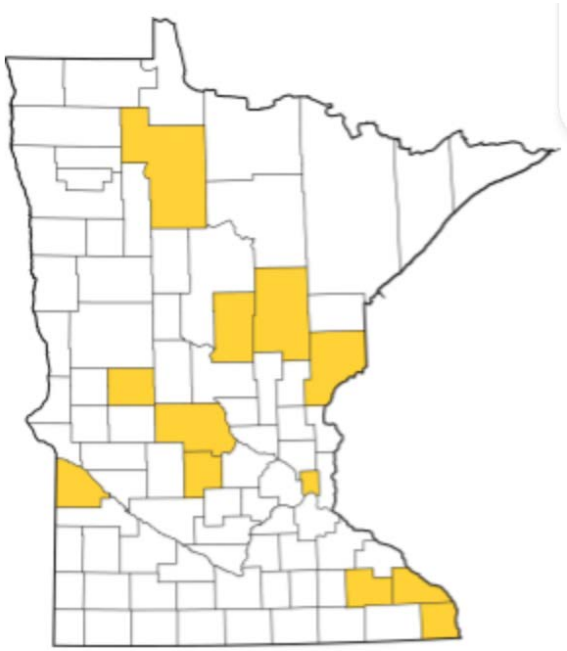
Michelle Carstensen



RT-QuIC analysis of over 3,165 datapoints (~790 lymph nodes)

RT-QuIC: opportunity for MN farmed cervid industry and state agencies?

- Beltrami Co. positive farm is furthest north in the state. Wild herd across state and tribal lands could be impacted.
- **We believe that our RT-QuIC-based diagnostics can be leveraged to help prevent farm-to-farm spread.**
- Must work with cervid industry, BAH, DNR, and UMN experts to effectively implement
- **MNPRO envisions a two-year pilot project to examine herd-level surveillance options:**
  - Use tank water, feces, soil to help detect prions early (similar to other livestock disease monitoring)
  - Ante-mortem testing using blood or saliva



Yellow counties with CWD positive farms since 2002.

Our team has developed a field-deployable 24-hour CWD test

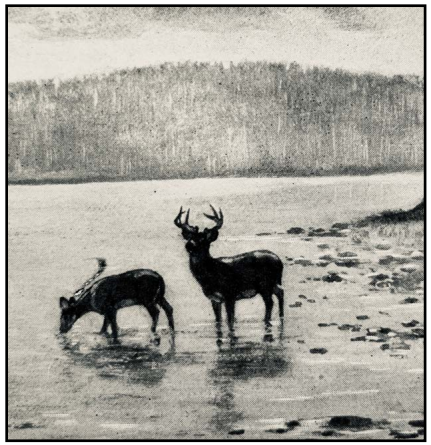
- Breakthrough in MNPRO lab occurred in October 2020
  - Performed dozens of follow-up experiments
- Feb 2021: determined we could deploy the prototype
- March 8th – 13th: deployed new test with DNR help in Rushford, MN
  - Day 1: confirmed that new test was working in the field
  - Day 2: successfully tested DNR tissues on site
  - Days 3 to 6: performed multiple confirmatory experiments
- Filing provisional patent (adhering to LCCMR and UMN policy). Writing manuscript to publish findings.

MNPRO: Moving Forward

- Continue to work with state, tribal, and national partners to advance CWD research and combat the spread of CWD

- MNPRO value system: uphold the tradition of research, service, and outreach associated with a land-grant institution

Thank you!



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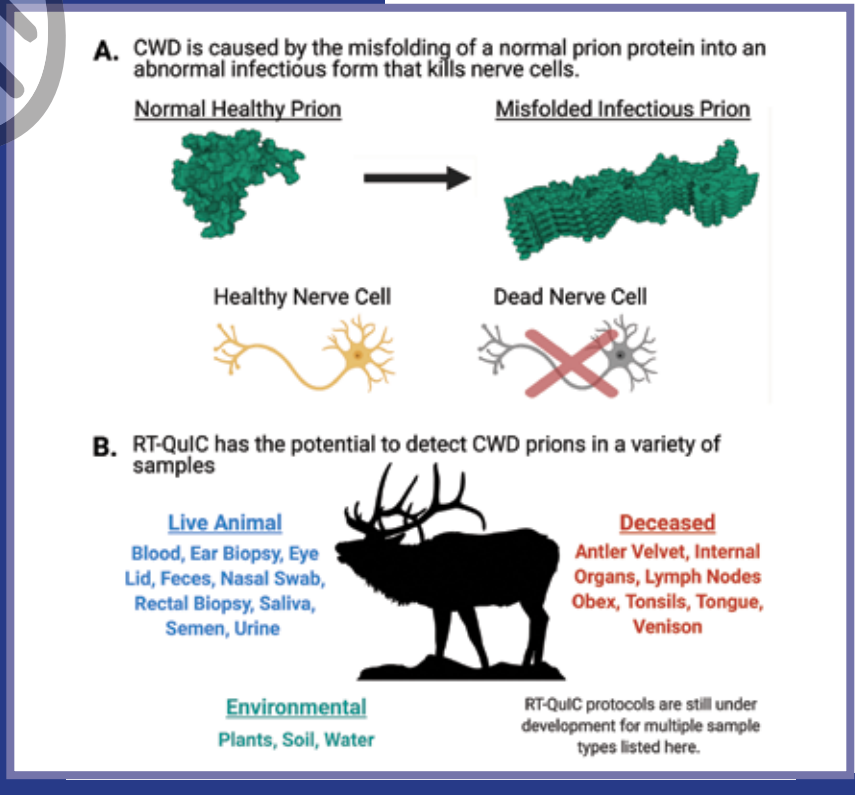
Attach. G

RT-QuIC: The Future of CWD Diagnostic Testing

By Peter A. Larsen, Ph.D., Department of Biomedical and Veterinary Sciences, University of Minnesota, and Minnesota Center for Prion Research and Outreach (MNPRO), University of Minnesota, St. Paul MN 55108

"Our research team is working to develop prion diagnostic tools that represent a generation beyond RT-QuIC."

Chronic wasting disease (CWD) of cervids is one of the most challenging animal diseases that we have ever faced, as scientists, producers, managers, and regulators alike. Since its discovery in Colorado in the 1960's, CWD has steadily spread across North America, leaving a destructive trail across cervid health and related economies in its wake. Decades of scientific research resoundingly shows that CWD is caused by pathogenic misfolded prion proteins, twisted molecules that ultimately clump together in cervid central nervous system tissues, forming plaques that are eerily similar to those observed in Alzheimer's, Parkinson's, and Huntington's diseases of humans. The shared features between CWD and other neurodegenerative diseases are not a coincidence, indeed, there exists an entire spectrum of protein-misfolding diseases in humans and animals that are related in different ways. CWD is part of that spectrum, it belongs to a family of prion diseases such as scrapie in sheep, bovine spongiform encephalopathy in cattle, Creutzfeldt-Jakob Disease in humans, and there are even prion diseases that infect camels, cats, and mink. The connections between CWD and other diseases of the nervous system cannot be ignored, because it is these connections that provide essential clues, threads of knowledge that will lead us to the development of new technologies to fight the war against CWD.



Fighting CWD requires tools that are sensitive enough to accurately diagnose the prion infection in both deceased and live animals, including animals that

Attach. H

have recently been infected. As with any infectious disease, the rapid and robust detection of the underlying disease agent is key to mounting an effective containment strategy. Existing CWD diagnostics (ELISA and IHC) are time-consuming, require expensive equipment, and cannot identify CWD during early stages of the disease. For these reasons, those who submit samples for CWD testing must endure long wait times before receiving the results and a "not detected" outcome might be inaccurate. All the while the disease continues its relentless march. It is abundantly clear that we must work urgently to develop new CWD diagnostic tools in order to protect the rich heritage surrounding cervids and all cervid-related economies. It is within the diagnostic realm that a spark of hope has emerged for all those impacted by the spread of CWD. Hope that traces its origin to a moment of scientific ingenuity in the mid 2000's. This hope, this new weapon in the war against CWD, is known as RT-QuIC.

It is within the diagnostic realm that a spark of hope has emerged for all those impacted by the spread of CWD.

Real-Time Quaking Induced Conversion (RT-QuIC)

In 2007, a research team working on the development of diagnostic assays for human prion diseases made a startling discovery. They could observe, in **Real-Time**, the misfolding of prion proteins from human Creutzfeldt-Jakob Disease samples. How did they accomplish this? As with all prion diseases (including CWD), a key feature of Creutzfeldt-Jakob Disease is the abnormal twisting of prion proteins that eventually begin to glob together (plaques) and kill brain and other nerve cells. The Creutzfeldt-Jakob Disease research team knew that if they could efficiently detect prion protein misfolding, then they should be able to accurately diagnose the prion disease. To accomplish this, they placed samples of misfolded prion proteins from Creutzfeldt-Jakob Disease-positive patients within tubes containing the normal, healthy version of the prion protein. They then warmed this mixture, shaked and **Quaked** it for a period of time. They observed that the misfolded prion proteins from the human patients, actively **Induced** the misfolding of the normal prion proteins, a domino effect that **Converted** them to the abnormal twisted form. They witnessed and measured this conversion using a fluorescent dye (thioflavin T), a molecular magnet that binds to misfolded prion proteins and that produces a light signal when beamed with a laser. This was the birth of RT-QuIC.

RT-QuIC as a Diagnostic Tool for CWD

The first scientific paper showing the potential for RT-QuIC as a diagnostic tool for CWD was published in 2010. Much like the original Creutzfeldt-Jakob Disease study, the CWD-focused experiment revealed that when a sample of CWD positive deer brain was placed in a tube with normal prion proteins under warming and shaking conditions, it caused them to misfold. The team monitored this misfolding using a machine that recorded the shape-change based on the same fluorescent magnet (thioflavin T), thus allowing for sophisticated statistical analysis to identify CWD positive samples. The RT-QuIC machine that is used to measure prion protein misfolding is incredibly sensitive and can detect tiny amounts of CWD prion proteins, opening the door for a wide variety of potential diagnostic applications.

Research papers using RT-QuIC are now routinely published, and the method has been used to detect CWD prion proteins within a wide variety of cervid samples, including: brain, obex, lymph nodes, tonsils, rectal biopsies, eye tissues, skin, blood, saliva, urine, and feces. Over the last year, our research team has performed thousands of RT-QuIC reactions using a variety of white-tailed deer tissues and our results show the method is not only 100% accurate when using the same tissues that the traditional CWD diagnostic tools use (lymph nodes), but it can also detect CWD

prion proteins that would have been missed using those traditional methods, such as in animals with early-stage infections. A number of research teams across the United States, including our own, are working diligently to internally validate RT-QuIC as a diagnostic tool for CWD using various tissues and biological samples while at the same time, the USDA is investigating the validation of RT-QuIC as a regulatory test.

Given the exceptional sensitivity of the method for detecting misfolded prion proteins, RT-QuIC is useful for screening both live and deceased cervids and cervid by-products. The method can be used not only on freshly deceased animals but also on animals that are found dead and are at varying stages of decomposition. A number of labs are currently developing protocols for RT-QuIC based detection of CWD prion proteins in plants, soil, water, and environmental surfaces. For these reasons, RT-QuIC has great potential to reduce additional spread of CWD. Another benefit of RT-QuIC is that the method can be scaled up to meet testing demands. The machines capable of performing the assay can analyze up to 128 samples (three replicates per sample) in a single run. Run-times for RT-QuIC largely depend on the sample type being analyzed. Detection of miniscule amounts of CWD prion proteins, such as those in feces, might take upwards of 48 hours, however, analyzing tissues where prion proteins concentrate (lymph nodes or brain) can be completed in less than 24 hours.

Current Limitations of RT-QuIC

Make no mistake, RT-QuIC is not a silver bullet for CWD and the method requires formal validation before it can be widely adopted for CWD diagnostics. Validation of a diagnostic test requires that the test be shown to routinely detect "true positives" and "true negatives" with a high degree of confidence. State-level validation of RT-QuIC can be performed within a certified diagnostic lab. However, when national-level regulatory policies are considered, the test must be validated and formally recognized by a national regulatory body, in this case the USDA. It is essential that state and national-level validation of RT-QuIC be performed before the test can be widely adopted for official CWD surveillance. Despite being ~14 years old, RT-QuIC is still a relatively new assay and only a handful of research and diagnostic labs have access to the method, thus slowing the validation process.

Another limitation of RT-QuIC is that the assay requires a special ingredient, recombinant "normal" prion protein that is cultured using bacteria within a lab. This ingredient is difficult to mass-produce and most labs grow their own recombinant prion protein for RT-QuIC. Thus, the ingredient is not widely available. Few institutions have the infrastructure required to mass-produce the recombinant prion protein required for RT-QuIC. This is a major limitation for national-level adoption of RT-QuIC and for this reason our team, and others, are working to solve the recombinant prion protein mass-production issue.

The Critical Need for Research Funding

Are the current limitations surrounding RT-QuIC insurmountable? No, they can be solved through the dedicated work of teams of scientists who are skilled at problem solving. Forming these scientific teams requires sustained funding that allows for the employment of laboratory technicians, purchase of necessary equipment for RT-QuIC, standardized production of the RT-QuIC ingredients, and purchase of the day-to-day consumables required to perform the test. Large grants from federal institutions that would specifically support CWD diagnostic research across the United States are lacking. For this reason, legislative bodies of individual states (such as Minnesota and Michigan) have stepped in to support the advancement of CWD diagnostic tests, including RT-QuIC. If we are going to win the war against CWD, we must have the ability to quickly and effectively identify the disease in both wild and farmed settings. This can only be accomplished through the formation of multidisciplinary research teams who are dedicated to solving the problem and who have the resources required to perform the necessary scientific

Attach. H

research. The recent forming of the multi-state North American Interdisciplinary Chronic Wasting Disease Research Consortium (supported by the USDA) is a positive step, as this effort unites over 40 scientists who will work together to solve a variety of CWD-related problems over the coming years.

A Path Forward

One of the most exciting aspects of the research surrounding RT-QuIC, is that it can be leveraged to develop next-generation diagnostic tools that are field-deployable and are cost-effective. These technical feats are not science fiction, they are already being developed for a variety of human neurodegenerative diseases and are driven by substantial federally funded research dollars. The story of RT-QuIC is common in science, important discoveries that are initially made based on well-funded human health research are then adapted and applied to a variety of areas. Advancements that focus on cutting-edge nanotechnologies for Alzheimer's, Parkinson's, and Creutzfeldt-Jakob Disease diagnostics can be modified to produce rapid and sensitive diagnostic tools for CWD. It is this area that holds great promise, and our research team is working to develop prion diagnostic tools that represent a generation beyond RT-QuIC. Adoption of any new technology for CWD diagnostics will take time, and there are those who question whether or not such advancements are even possible. Failure is not an option. We must think outside of the box, leverage our collective strengths, and do all that we can to protect the cervids of North America.

Dr. Peter Larsen is an Assistant Professor in the Veterinary and Biomedical Sciences Department of the University of Minnesota College of Veterinary Medicine. Dr. Larsen is Co-Director of the Minnesota Center for Prion Research and Outreach (MNPRO). He leads a diverse research program focused on the discovery of emerging infectious diseases, field-based molecular diagnostics, and the mechanisms of neurodegenerative disease.

Please visit the MNPRO website for additional information: **https://mnpro.umn.edu/**

MN Minnesota

MI Michigan

"...legislative bodies of individual states (such as Minnesota and Michigan) have stepped in to support the advancement of CWD diagnostic tests, including RT-QuIC."

Attach. H

Texas Ingenuity and Live Animal CWD Testing

Interview with Patrick Tarlton, *Texas Deer Association, Executive Director*

Written by Brenda Hartkopf, NAEBA Staff

Texas is one of many states that has dual state agency oversight of farmed cervids. One state agency is the Texas Animal Health Commission and the other Texas Parks and Wildlife. The amazing part of this story is the work these two state agencies have done along with industry, to create an "outside the box" CWD testing protocol to keep producers in business who could not comply with the federal program or suddenly find themselves with a CWD positive animal on their ranch. In the process, Texas has accumulated over 120,000 live animal test results on farmed whitetail deer since 2016! This has created a vast test sample database that would be virtually impossible to recreate through any other resource.

CWD was first found in Texas in a captive facility in 2015. Upon the discovery, both the USDA APHIS and wildlife programs were intensely scrutinized. Up to that time, Texas Animal Health Commission utilized the USDA CWD Program Standards, but Texas Parks and Wildlife utilized a unique program for farmed whitetail deer where for intrastate movement in Texas, a breeder was only required to CWD test one of every five animals that died, for a total of 20% surveillance. Upon the first CWD finding, a joint task force and overseers of permits created emergency rules, interim rules and issued an executive order which all created intense hardship on the industry.

When the sudden executive order went into effect, it forced breeders to enhance their level of CWD testing from previous years. Now instead of testing 20% of their mortalities, 80% of mortalities needed to be tested retroactive to the five previous years. Obviously, there was no going back in time. Therefore, to make up for any missed tests, a certain number of live animals had to be killed and tested to get the testing level up to 80% retroactively. This sent the industry into an uproar. Everyone was fighting; even legislators became involved because they had people in their district suddenly being forced to kill perfectly healthy animals in order to stay in business.

CWD Live tests
completed on farmed whitetail deer to date:
120,000+

In 2016, newly formed CWD Management Rules were developed between the state agencies and industry, which included a live testing provision. This had never been done anywhere in the country; Texas now became the first state to have CWD regulatory surveillance based in part upon live animal testing. This applied only to whitetail deer and no other cervid species and would be acceptable for intrastate movement only.

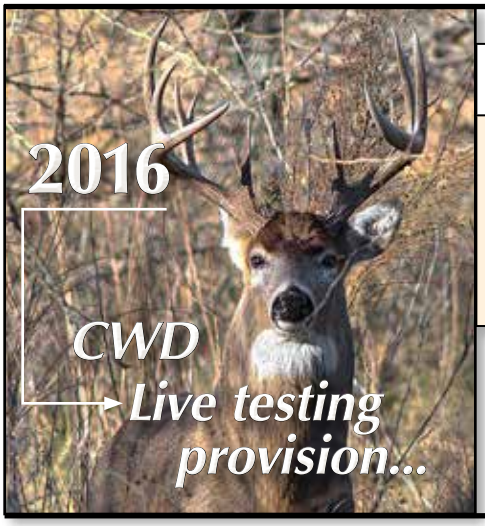
From 2016 forward, Texas whitetail breeders have had to test 80% of animal deaths in their herds to comply with the new program. Additionally, each ranch needs to test a minimum of 3.6% of age eligible animals, 12 months and older, each year. If a producer does not catch the minimum of 80% of deaths to test, they are allowed to live test three animals for every one animal missed. This is referred to as the "3-1 ratio". If a herd has not reached testing 3.6% of their age eligible animals in a given year, they can also live test three animals for every animal needed to reach 3.6% of the herd tested. This is determined every year at annual reporting time which is March 31. On April 1, every ranch has to determine if they need to live test additional animals.

When conducting live testing for surveillance purposes, using either the rectal or tonsil test is acceptable. When a CWD positive is found, then only the tonsil test is utilized as it is thought to be the more sensitive live animal test of the two.

Even though this program is for Intrastate movement (within the state) only, trace-in and trace-out program administration is done by the Texas Animal Health Commission who also determines the herd plan moving forward. Testing regiments have been created that can be used in certain situations to prevent long term lockdowns. Once a positive is found on a facility or if a facility becomes linked with another positive facility, both state agencies come in and tonsil test every animal. If another positive is found, all pen mates to the positive animal are euthanized. Among other requirements, they segregate the rest of the deer away from the infected pen, wait five years and are required to whole herd tonsil test every third year. When depopulating with partial kills, no indemnity payment is offered, but in the case of full depopulation of the herd, indemnity is usually available.

There has been no CWD live testing regimen available in Texas for other susceptible cervid species such as elk. But CWD has not been found in any other breeding facilities besides whitetail deer in the state either. Plus, Texas Parks and Wildlife only regulate

Attach. I



YEAR	CWD TEST REQUIREMENTS Due March 1st		
Since 2016	**ANNUALLY** 80% of animal deaths need to be tested for CWD	A minimum of 3.6% of age eligible animals in the herd, 12 months and older, must be tested each year	**3-1 RATIO** If 80% minimum is not met, allowed to live test three animals for every one animal missed and a minimum of 3.6% of the herd has been tested
			Either the rectal or tonsil test is acceptable for regular surveillance purposes

whitetail and mule deer in the state of Texas. Elk, red deer and other cervids are under oversight of the Texas Animal Health Commission.

With 120,000+ live CWD tests completed to date, this is an incredible number of tests from which to advance science. They have determined that enough tests have been conducted to feel confident this form of testing creates adequate sensitivity and that they can be confident in animal movement and adequately survey their state.

Many times, one will hear that if a CWD test is not a 100% accurate test, then it is no good. The fundamental difference between what Texas is doing and this thinking, is that they are not determining the test regiment by a single animal, but rather, by the ranch as a whole. If a ranch is sufficiently tested, and an area sufficiently tested, then there is no reason live testing is not adequate.

Looking at the breeder facilities in Texas, they feel live testing has been adequate. Compare this test regimen to every other animal disease in America such as TB, etc.- none are 100%! The tonsil test in one whitetail deer is more sensitive than a Coggins test in a horse and horses are shipped everywhere across state lines in the United States. Zero risk is appreciated, but where are all the other programs that show 100% accuracy? There are none.

TEXAS
1st State to use CWD live testing as part of regulatory surveillance.

As the science becomes more accurate and valid, it keeps the industry in business.

• • •

With the live animal testing that has been accomplished here, more information has been added to the science. Anything that helps a producer alleviate risk ought to be considered.

Looking back at all the things learned about CWD in Texas since 2015, these things stand out as surprises to Patrick:

1. We are still learning a lot about CWD and there's a lot left to learn.
2. Sometimes industry is too quick to believe in something before there is enough research. For instance, some DNA markers thought to provide CWD resistance in whitetail deer do not seem to be as valuable as we thought.
3. Many thought live testing would be the death of the industry based on cost and lack of experienced veterinarians, but we found the opposite to be true. The industry was driven to find a solution. This testing has made the industry really strong. Producers can find a way to test their animals to get out of a lockdown situation. This is huge.

To date, Texas has found CWD in five ranches. One facility was a breeder operation and killed all the whitetail deer. They now raise fallow deer who have been deemed resistant to CWD and are very marketable in Texas. Another positive facility had the breeding and ranching facility for a while but are now just releasing bucks for hunting. The other three facilities still allow hunting on their property. Every animal that is released on these still operating hunting facilities must be tonsil tested prior to release in compliance with the program.

It's not shocking to have found CWD in Texas as it is present on the state borders with New Mexico and nearby Colorado, and now present in a few central Texas deer breeder facilities. However, live CWD testing has saved Texas whitetail deer markets. It truly allows breeders to move animals at times of business risk.

Patrick Tarlton is the Executive Director of the Texas Deer Association.

Attach. I

Surveillance and Management Plan for Chronic Wasting Disease in Free-ranging Cervids in Minnesota

July 2019

Attach. J

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Introduction

Chronic wasting disease (CWD) belongs to a family of infectious diseases, called transmissible spongiform encephalopathies (TSE), which alter the morphology of the central nervous system, resulting in a "sponge-like" appearance of this tissue. Chronic wasting disease affects members of the cervidae family including elk (*Cervus canadensis*), mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), moose (*Alces alces*), and caribou/reindeer (*Rangifer tarandus*). The causative agent of CWD is an infectious protein, called a prion, which accumulates in nervous and lymphatic tissues and has been detected in a variety of tissues and fluids throughout infected animals. Infectious prions have been found in cervid urine, saliva, blood, feces, muscle, and antler velvet (Mathiason et al. 2006, Haley et al. 2011, Pulford et al. 2012). Incubation time of the disease, from infection to clinical signs, can range from 1.5 to nearly 3 years (Williams et al. 2002). Clinical signs are non-specific and may include a loss of body condition and weight, excessive salivation, loss of fear of humans, loss of body control, tremors or staggering, drooping head or ears, and apparent confusion (Gilch 2016). There is no known treatment or vaccine for the disease and it is always fatal.

Chronic wasting disease was first discovered in captive mule deer in 1967 in Colorado and then recognized in captive white-tailed deer and elk in 1978. Within wild populations, CWD was historically confined to free-ranging deer and elk in the endemic area of northeastern Colorado and southeastern Wyoming. As of fall 2018, the disease has been detected in wild and captive cervids in 25 states across the US, three Canadian provinces, the Republic of Korea, Finland, and Norway.

The Centers for Disease Control (CDC) and other public health agencies have concluded there is no known link between CWD and any neurological disease in humans (MaWhinney et al. 2006), and transmission to humans is extremely unlikely (Kurt et al. 2015, Waddell et al. 2018). However, an ongoing study by the Canadian Food Inspection Agency has demonstrated that by orally consuming muscle from deer naturally infected with CWD under experimental conditions, the disease can be transmitted to macaques (*Macaca fascicularis*). This unpublished finding has sparked renewed concerns about potential human health risks of eating CWD-contaminated venison (Czub 2017). In separate work, also focused on susceptibility of macaques to CWD, Race et al. (2018) found no evidence of successful transmission. The reasons for this

scientific ambiguity are unclear, but as a precaution both the CDC and the World Health Organization recommend that humans do not consume any part of a known positive animal.

Experimental and circumstantial evidence suggest that transmission of the disease is primarily through direct contact with infected animals, carcasses, saliva, or excrement (Miller and Williams 2003, Safar et al. 2008, and Haley et al. 2011). There is also evidence that CWD can be transmitted vertically from mother to offspring (Nalls et al. 2013). Theoretically, prions can be shed from infected animals soon after initial infection; in one experimental study prion shedding was detected in deer saliva three months post-inoculation (Henderson et al. 2015), and another study found that deer shed prions in their feces up to a year before showing signs of illness (Tamgüney et al. 2009).

Persistence of prions in the environment and resulting indirect transmission has been shown to occur (Miller et al. 2004, Johnson et al. 2007, and Maluquer de Motes et al. 2008). Prions readily bind with soil particles (Saunders et al. 2012) and other abiotic substances (Pritzkow et al. 2018), which can magnify CWD infectivity (Johnson et al. 2007). Furthermore, plants have been shown to uptake prions from the soil making them available for consumption by herbivorous animals (Pritzkow et al. 2015). Conversely, a recent study found that humic acids in soil organic matter may decrease prion infectivity (Kuznetsova et al. 2018). These findings underscore the complex dynamics that prions have with the environment.

All cervids infected with CWD, regardless of their genetic makeup, will die from CWD-associated mortality because no genotype confers complete immunity. However, research has demonstrated that certain genotypes can extend the CWD incubation period and animal survival time, although these infected individuals may shed infectious prions for a longer amount of time (Johnson et al 2006, 2011; Robinson et al. 2012). Based on epidemiological modeling, deer with a more CWD-resistant genotype may have a selective advantage in the long term, although it is not clear if there are maladaptive traits associated with their presence (Robinson et al. 2012). More recently, there is evidence that there has been genetic selection among elk due to CWD, but it is unclear whether it is sufficient to mitigate negative population level impacts (Monello et al. 2017). There is much uncertainty regarding how CWD may drive the evolutionary dynamics of cervid populations, but it is clear that the recent discovery and potential for novel CWD strains adds additional complexity (Duque-Velásquez et al. 2015).

White-tailed deer have significant cultural, social, and economic value in Minnesota. These values extend particularly to Tribal communities on both Reservation lands and Ceded Territories where cervids provide sustenance for those communities. On these lands especially, close coordination with Tribal partners on all aspects of CWD response activities is critically important. As written in the MNDNR White-tailed Deer Management Plan, agency staff coordinate and work with tribes on deer management in accordance with reserved treaty rights, associated court decisions, federal laws, intergovernmental agreements and shared interest in natural resource conservation. In 2019, Governor Tim Walz signed Executive Order 19-24 with the intent to improve relationships and coordination with tribal nations. The MNDNR acknowledges that this disease may negatively affect treaty resources and influence the ability of tribal members to exercise their treaty rights; close coordination is imperative as specific response plans are developed on reservation lands and ceded territories.

Throughout Minnesota, hunting activities related to wild white-tailed deer generate over 500 million dollars for Minnesota's economy, and drives the economic engine for the Minnesota Department of Natural Resources (MNDNR), Division of Fish and Wildlife. The discovery of CWD in wild cervid populations has negatively affected hunter numbers and local economies in areas where it exists (Vaske et al. 2004, Vaske and Lyon 2011). In fact, deer license sales in southeast Minnesota have declined 10% since 2016, which is inconsistent with prior year sales that were relatively stable. If CWD were to become established or if the disease is determined to impact human or domestic animal health, the MNDNR would realize substantial reductions in license sales and Federal Aid reimbursements and negatively affect the agency's ability to manage all wildlife in the public trust. Needham et al. (2004) postulated that upwards of two-thirds of hunters would quit hunting if CWD was transmissible to humans. By extension, a reduction in deer hunters diminishes the capacity of state wildlife agencies to effectively manage deer populations. The MNDNR is not unique, as license fees fund the operations of most state wildlife agencies, who are reliant on these fees and Federal reimbursements to deliver management and conservation activities for many species of wildlife and their habitats (Organ et al. 2010). Since 2002, Minnesota has spent $8.4 million on its CWD response program[1], of which 96% were state funds (83% from license fees). Until such time as uncertainty is reduced

[1] Fiscal year 2019 ($1.4 million) in an estimate.

(e.g., human health implications, long-term population concerns), the MNDNR should consider CWD response/management as the highest priority and all necessary resources should be directed to avoid the long-term consequences of an endemic infection.

History of CWD in Minnesota

To date (June 2019), CWD has been diagnosed in 8 captive cervid herds within the state of Minnesota, including 3 elk herds, 4 white-tailed deer herds, and 1 European red deer (*Cervus elaphus*) herd. Two of the elk herds (Stearns and Aitkin counties) were discovered in 2002 and depopulated with no additional CWD positive animals found. In spring 2006, a captive white-tailed deer was found infected with CWD from a mixed deer/elk herd in Lac Qui Parle County. That herd was also depopulated without additional CWD cases being detected. In early 2009, a third captive elk herd (Olmsted County) of >600 animals was found infected with CWD. Following depopulation, a total of 4 elk were confirmed with the disease and the United States Department of Agriculture (USDA) determined there was an apparent longstanding infection within the herd. In mid-2012, a captive European red deer was found infected with CWD in a herd of approximately 400 animals in North Oaks (Ramsey County). This marked the first time CWD was discovered in this species (Schwabenlander et al. 2013). Also in 2012, the USDA discontinued funding to depopulate CWD-infected herds; thus the North Oaks herd was quarantined for several years until depopulation finally occurred in 2015 (with no additional CWD positive cases found). In 2016, two adult female white-tailed deer were detected with CWD from a captive mixed white-tailed and mule deer herd in Crow Wing County. This facility remained under quarantine as the owner chose not to depopulate the herd; it continued to operate as a shooting pen, although CWD was again detected in this facility in fall 2018. Movements from this facility to a herd in Meeker County in 2017 revealed another infected adult female white-tailed deer, which was found dead in the facility and confirmed to have CWD. This herd of 15 deer was depopulated with 5 animals (33% prevalence) testing positive for CWD. Most recently, in November 2017, CWD was detected in an adult male white-tailed deer that died in a Winona County captive deer facility. An additional adult male harvested in the facility in December 2017 was also confirmed infected with CWD. The remaining seven animals in this facility were depopulated in early 2018 and all deer tested positive for CWD (100% prevalence). The Crow Wing farm was depopulated in spring 2019 and 7 additional deer were identified as

positive from 89 deer sampled. On-farm prevalence could not be determined because 13 of 20 found dead deer on the farm were not tested.

In response to the initial discovery of CWD in wild white-tailed deer of Wisconsin and the first Minnesota CWD-positive captive elk herd in 2002, the MNDNR developed a comprehensive wild deer CWD surveillance program. This included surveillance of hunter-harvested and opportunistically encountered vehicle-killed and clinical-suspect deer, elk, or moose. A clinical-suspect cervid is defined as exhibiting physical signs, behavioral abnormalities, and/or locomotor difficulties consistent with CWD infection (Gilch 2016). From 2002–2004, nearly 28,000 deer were tested for CWD statewide with no positive results. Sampling occurred at the deer permit area (DPA) level with 95% confidence that the disease would have been detected if present in ≥1% of the deer population in each DPA. Following completion of statewide surveillance, and no evidence of a long-standing infection in Minnesota, the MNDNR adjusted surveillance efforts and focused sampling of wild cervids in response to elevated risk factors. These risk factors include 1) detection of CWD-positive animals in a captive cervid facility in Minnesota, 2) proximity of positive CWD cases in wild deer in neighboring states, and 3) testing of clinical-suspects of CWD and other special wild cervid cases. These elements constitute the MNDNR's risk-based approach to CWD surveillance in Minnesota and permit more efficient use of finite resources (financial and personnel), as opposed to continuous statewide sampling. Since 2005, the MNDNR has tested an additional 43,000 deer for CWD using risk-based surveillance.

The first wild white-tailed deer found infected with CWD in Minnesota occurred in fall 2010, during the second year of risk-based surveillance efforts surrounding the CWD-positive captive elk facility in Olmsted county in 2009. From 2011–2013, MNDNR implemented the 2011 CWD Response Plan (MNDNR 2011) and over 4,000 deer were tested in this immediate area; no additional positives were detected (Hildebrand et al. 2013). The MNDNR concluded the disease was either found early enough to prevent establishment or occurred at an undetectable level in the local deer population. The CWD Management Zone that had been created through the response plan was dissolved in 2014, and harvest regulations and zone boundaries returned to what they were prior to the discovery of CWD.

In fall 2016, surveillance efforts were again prompted by a risk-based approach as detections of CWD in wild deer from western Wisconsin and northeastern Iowa increased and further encroached on Minnesota borders. As a result of this effort, three adult males were found infected with CWD in Fillmore County (DPA 348). Again, MNDNR implemented the 2011 CWD Response Plan and additional samples were collected during winter 2016-2017, which resulted in eight more CWD-positive deer found in a small geographic area near Preston, Minnesota. Initial disease prevalence was estimated at 0.7% within the newly established CWD Management Zone (DPA 603), with nearly all CWD cases found within a 64mi^2 core area (2.1% sample prevalence). Testing within DPA 603 during fall 2017 resulted in six additional CWD-infected deer and sample prevalence was estimated at 0.4%, with possible disease spread approximately 8 miles west of the core area into Forestville State Park. Surveillance efforts in fall 2018 detected an additional 17 CWD-positive deer within DPA 603, increasing our sample prevalence to almost 1% (0.98) within the CWD Management Zone. Further, the first cases of disease were found outside the DPA 603 boundary in 2 deer in DPA 347 and 4 deer in DPA 346. To date, a total of 50 deer have been confirmed to have CWD in southeastern Minnesota since 2016. Ongoing surveillance and aggressive management in DPA 603 will determine if MNDNR's response actions were successful in reducing or eliminating CWD, or if the disease will continue to persist and require adaptive management responses.

In late-January 2019, an emaciated deer was found dead 0.5 miles from a CWD-positive captive cervid facility in Crow Wing County; the deer (adult female) was confirmed to have CWD. This was the first wild deer detection in an area that has been under DNR's risk-based surveillance program since 2017, after the facility was found infected in December 2016. Immediate steps through spring 2019 included working with landowners to remove wild deer from around the facility in order to remove potentially positive animals from the population; 115 additional deer were sampled and none were positive for CWD. A new disease management zone will be established in the area by fall 2019.

Risk-based Surveillance for CWD in Wild Cervids of Minnesota

The MNDNR goal for CWD surveillance in wild cervids is to detect the disease, if it is present, as early as possible since introduction. Since 2005, the MNDNR has relied on a risk-

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Current Scientific Knowledge About CWD

April 2018

American Cervid Alliance Introduction

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American Cervid Alliance Introduction

In an ongoing effort to keep the public apprised of the latest in scientific facts as they relate to Chronic Wasting Disease, the American Cervid Alliance is endorsing the following scientific paper prepared by Dr.'s Don Davis, James Kroll, Greg Stewart, and Ken Waldrup, which dispels much of the mythology surrounding CWD.

This well-prepared paper, sponsored by the ACA, uses science-based facts, giving the reader a clear view of what is known and not known about the disease in contrast to what some are merely theorizing about CWD by using non-scientific opinions, theories, and beliefs to further a biased agenda.

We welcome you to share this document with your legislators and wildlife or animal health officials, as well as members of the media and the public to give a more accurate perspective about a disease that is affecting all segments of deer and elk populations.

Attach. J

Basic Facts Surrounding CWD

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Basic Facts Surrounding CWD

A

If an individual deer of a species susceptible to CWD is exposed to a sufficient number of infectious CWD prions, morbidity and mortality may be induced after a prolonged incubation period of 17 months to more than 4 years. After the onset of clinical signs caused by spongiform encephalopathy (holes in the brain) the disease is usually fatal and rapidly so.

B

CWD is a fairly rare disease with a prevalence less than 1% in the over million deer tested nationally over the last 20 years, and a prevalence of 11.2% in the 196 CWD positive counties in the 23 CWD positive States. After 30 years, the CWD test positive prevalence rates in a few states have been reported to be 35-40%. Actual data shows much less. CWD test positive prevalence is an indication of infection and exposure, but CWD test positive prevalence is not a measure of and does not equal mortality from CWD.

C

CWD has continued to be found in new areas since the 1960's. This is a function of increased surveillance testing, natural animal movement, commercial transportation of animals, and the occasional spontaneous genetic mutation of the CWD prion.

D

In spite of the expenditure of over $100,000,000 of public funding, and thousands of animals killed, none of prevention, control, or eradication methods employed by the various States since 1998 have been shown to be effective in either preventing increased prevalence of CWD or the increased geographic distribution.

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Basic Facts Surrounding CWD

E

CWD is neither a “wild deer” disease nor a “captive deer” disease but can be found in both. There are 3 States with CWD only in captive deer herds and 8 States with CWD only in wild free-ranging populations. Based on USDA positive test prevalence numbers, CWD is more common in wild cervids than in captive cervids.

F

In small populations in localized areas of Wyoming, CWD may possibly be a factor along with many other factors in causing population declines. Deer populations in the Western States have been declining at 18-20% for over a decade in both states with CWD and those without CWD. Wildlife agencies report that habitat fragmentation, habitat loss, severe weather (droughts and bad winters), human disruption (oil exploration, real estate development), malnutrition, and predation are thought by biologists to have more influence on populations than disease (all disease including EHD, parasites, and CWD).

G

Predictive computer simulation models are just predictions not known facts. They are based on currently available information or assumed information on many variables. If either new scientific data becomes known or conditions such as climate change in the future, then the predictions generated by the model become invalid.

H

Since CWD primarily is a frequency dependent disease in wild deer instead of a density dependent disease, and the benefits of supplemental feeding in most cases far outweigh any possible problems associated with crowding. There is no published scientific data regarding the risk of CWD transmission associated with supplemental feed.

Attach. J

I

The exact modes of CWD transmission in wild deer are unknown. The numbers of CWD prions shed by infected deer in natural conditions is unknown. The length and timing of CWD shedding by infected deer is unknown. The genetic effects on CWD susceptibility and resistance to infection are unknown in susceptible species. All the above unknowns should be given an increased research priority.

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Table of Contents

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I. General Concepts And Definitions

Chronic Wasting Disease (CWD) is well-known Transmissible Encephalopathy (TSE) of several species of Cervidae or the Deer Family primarily found in North America. Centers for Disease Control (CDC) defines CWD as follows:

"Chronic wasting disease (CWD) is a prion disease that affects deer, elk, reindeer, sika deer and moose. It has been found in some areas of North America, including Canada and the United States, Norway and South Korea. It may take over a year before an infected animal develops symptoms, which can include drastic weight loss (wasting), stumbling, listlessness and other neurologic symptoms." CDC Aug 2017

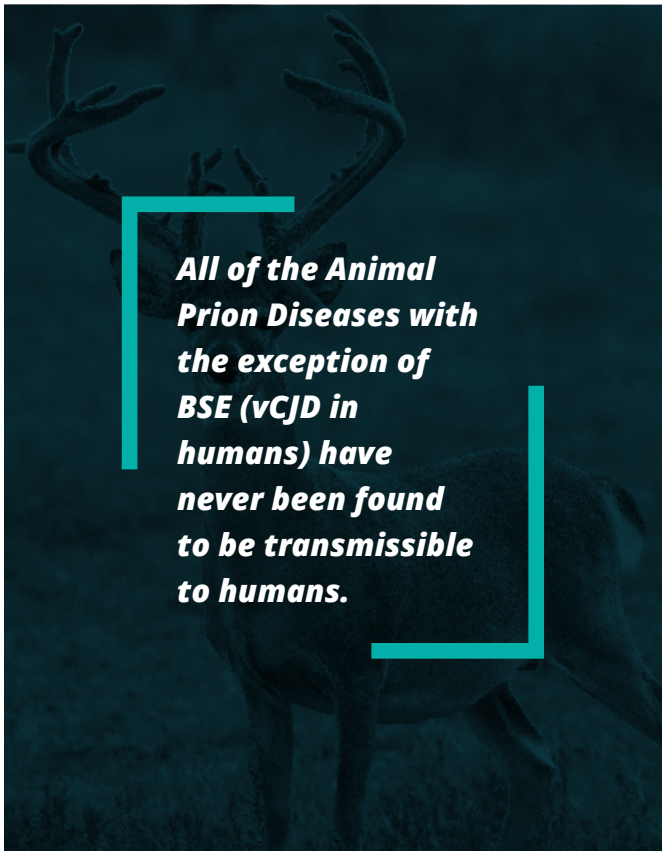
Note: CWD has very recently been diagnosed in the remains of a 15 year old European Elk (moose) in Finland. https://yle.fi/uutiset/osas/news/first_case_in_finland_elk_dies_due_to_chronic_wasting_disease/10108115

The etiologic agent of CWD and other TSEs based on available data is thought to be prions. Prions are self-replicating proteins and are found in their natural structure in normal animals and humans. An atypical structured prion causes pathologic changes in the susceptible host.

"Prion diseases or transmissible spongiform encephalopathies (TSEs) are a family of rare progressive neurodegenerative disorders that affect both humans and animals. They are distinguished by long incubation periods, characteristic spongiform changes associated with neuronal loss, and a failure to induce inflammatory response." CDC Aug 2017

"The causative agents of TSEs are believed to be prions. The term "prions" refers to abnormal, pathogenic agents that are transmissible and are able to induce abnormal folding of specific normal cellular proteins called prion proteins that are found most abundantly in the brain. The functions of these normal prion proteins are still not completely understood. The abnormal folding of the prion proteins leads to brain damage and the characteristic signs and symptoms of the disease. Prion diseases are usually rapidly progressive and always fatal." CDC Aug 2017

There are a number of TSEs that occur in humans and animals.



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Human Prion Diseases

- Creutzfeldt-Jakob Disease (CJD) (found in 1 per million worldwide, WHO)
- Variant Creutzfeldt-Jakob Disease (vCJD) (Total worldwide 229 cases)
- Gerstmann-Straussler-Scheinker Syndrome
- Fatal Familial Insomnia
- Kuru

Animal Prion Diseases

- Bovine Spongiform Encephalopathy (BSE)
- Chronic Wasting Disease (CWD)
- Scrapie (sheep)
- Transmissible mink encephalopathy
- Feline spongiform encephalopathy
- Ungulate spongiform encephalopathy

Note; All of the Animal Prion Diseases with the exception of BSE (vCJD in humans) have never been found to be transmissible to humans. This is discussed in detail below in other sections.

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II. Host Distribution

Naturally Occurring in Free-Ranging

Mule Deer (*Odocoileus hemionus*)

Rocky Mountain Elk (*Cervus elaphus nelsoni*)

White-tailed Deer (*Odocoileus viginianus*)

Shiras Moose (*Alces alces shiras*)

NOTE: More recently in Reindeer (Rangifer spp) and European Elk (Alces spp) in Norway and European elk remains in Finland.

Other Susceptible Cervidae (Deer Family)

Sika Deer (*Cervus nippon*) one in Korea in captive situation with other imported animals.

Red Deer (*Cervus elaphus*) – in captive and experimental infections

Muntjac deer (*Elaphodus muntiacus*) – experimental infection

NOTE: Fallow deer (Dama dama) resisted attempts to infect them for 7 years by USDA. Axis deer (Axis axis) have been tested in surveillance programs without finding any positives.

NOTE: There has been speculation since the 1980's about possible transmission to humans. To date, there has NOT been a single case of CWD in humans. This is discussed in detail in later sections.

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III. Ocurrence And Geographic Distribution

CWD was first observed as an unnamed clinical syndrome in 4 captive mule deer and elk groups of wild origin at Colorado State University (CSU) in the 1960's. (Spraker). That fact does not imply or in any fashion confirm that CWD originated in captive deer at CSU. There are several theories about the possible origins of CWD and all are not proven and never will be. The same may be said about the origins of BSE, scrapie, CJD, or any of the other naturally occurring TSEs.

The best review of the early cases of CWD at CSU and the Early History of CWD can be found in the "Chronic Wasting Disease: A Review for Health Canada' 105 pages by Dr. Terry Spraker from CSU.
The first published article on CWD was by Williams and Young in 1980 in the Journal of Wildlife Disease titled "Chronic Wasting Disease of Captive Mule Deer: A Spongiform Encephalopathy."

In 1981, CWD was found in a free-ranging 18 month old male elk in the Rocky Mountain Park. Then CWD was found in a 4-5 year old mule deer buck near CSU in 1984. The first case of CWD in a white-tailed deer was found in 1985 in a wild adult male near Loveland Colorado.

Now after more than 40 years, the rest, as they say, "is history". Presently as of Feb 2018, CWD has been found in 23 States and 2 Canadian Provinces, in Korea, Norway, and Finland. Uninformed and misinformed pundits often characterize CWD as a "common", "widespread" and/or "rapidly expanding" disease. A closer examination and even casual glance at the available scientific data refutes those claims.

According to CDC in 2012, "More than1,060,000 cervids have been reportedly tested for CWD, and ~6,000 cases have been identified." (Prevalence of positives = 0.56% which is 5 test positives per 1000 tested). USDA Records from 1998-2012. Dr. Patrice Klein USDA/APHIS on April 2012.

Total Farmed Surveillance
170,120 403 positives (0.2%)

Total Wild Cervid Surveillance
848,706 3,600+ positives (0.4%)

Total Tested
1,018,826 4,003 positive (0.39%)

Data from the Texas Veterinary Diagnostic Laboratory (TVMDL)

From 2013-2018 98,524 deer tested for CWD, with 87 positives (0.88%)

CWD Distribution By Counties

In the US, there are 3,144 Counties. As of February 2018 there are 196 Counties with CWD Positive Deer (CDC Jan 2018). That equals to 6.2% of the Counties in the US with CWD. That also means 93.8% of the US is free of CWD.

In the 23 States (counting Mississippi) that have CWD, there are 1,714 Counties. So according to CDC in January 2018, there

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Ocurrence And Geographic Distribution cont.

are 196 counties with CWD positive animals in the 1,714 total counties in the 23 CWD positive States. That equals 11.4%. Which means that even in the 23 CWD Positive States 88.6% of the counties are free of CWD as on February 2018, and some only have one positive.

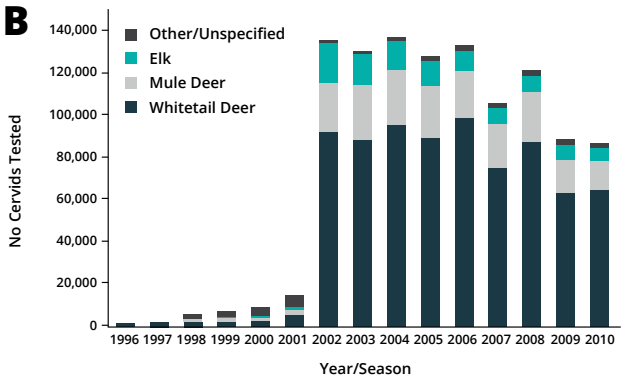
CWD Test Positive Prevalence – SCWDS Briefs. January 2018. Michigan since 2015, 1.9 % (57/30,000); Missouri 2016-2017, 0.24% (58/323,456); Nebraska since 1997, 0.99% (499/51,000); Wisconsin, since 1999, 1.99% (4174/209,700). Wyoming Game and Fish, January 2018, in 2017, 8.8%, 342/3883).

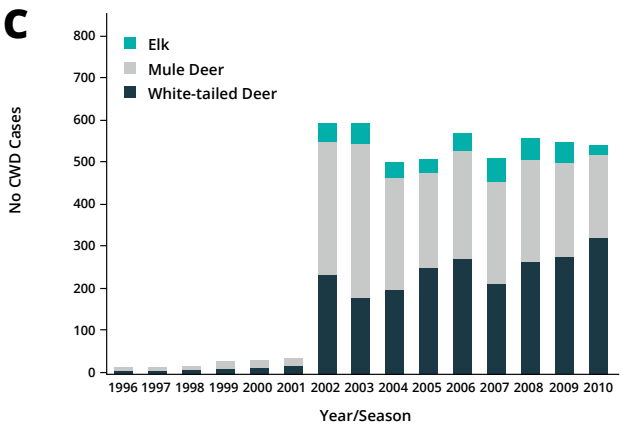
NOTE: The numbers above from SCWDS do not even approach the exaggerated prevalence numbers frequently and widely reported in the popular media sources.

It must be pointed out that the entire US and Texas do not entirely represent the CWD situation in all of the individual States. Wyoming, for example, has been reported to have a 35% prevalence of CWD in tested animals, there are CWD test positive animals in 16 of the 23 Counties in Wyoming, and the disease has been there for about 40 years. Wisconsin has recorded CWD in 20 of the 72 counties from 2002-2018. But the CWD situation in Wyoming and similar States should not be extrapolated to or used to make policy decisions in other States.

The five states of Wyoming (16/23), Colorado (20/64), Wisconsin (20/72), Nebraska (35/93) and Kansas (22/105 have 113 of the 196 or 58% of the CWD infected counties. There are 15 States that have less than 10 Counties each with CWD.

DATA from CDC that clearly shows that CWD is NOT Increasing in Prevalence Since 2002 When Surveillance Drastically Increased





Bottom Line on Occurrence and Distribution

Prevalence rates of less than 1% for CWD, like all the other TSE, shows that CWD is a fairly rare disease on a national scale. "Widespread" also depends on the scale by which it is measured. By total State is 44%, by Counties in the US it is 6.2%, by positive counties in the 23 positive states it is 11.2%.

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Even with the dramatic increase in surveillance and the number of deer tested since 2002, the prevalence has not increased nationally, however the increase in the number of States with CWD can be attributed to: 1) the natural movement of deer, 2) the transportation/translocation involved in deer commerce, and 3) the increase in required CWD testing.

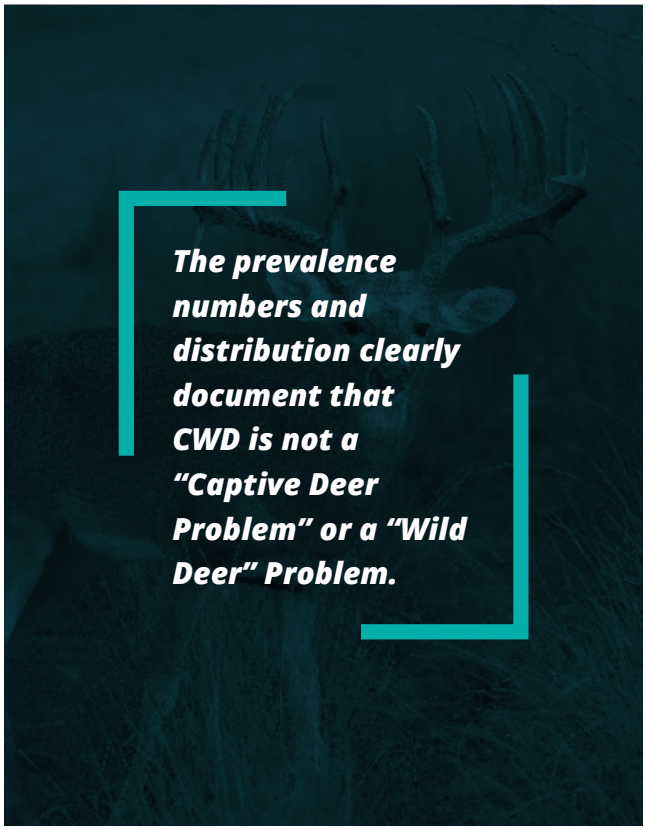
It should also be noted that some of the "spread" of CWD could be cases due to spontaneous mutations. All TSEs have spontaneous cases of atypical forms of prions. Diagnostic testing for Spontaneous CWD is rarely done. This will be discussed at length in TRANSMISSION.

NOTE: The prevalence numbers and distribution also clearly document that CWD is not a "Captive Deer Problem" or a "Wild Deer" Problem.

According to USDSA/APHIS, "since 2001, CWD has been identified in free-ranging cervid populations in 23 States: Colorado, Illinois, Kansas, Maryland, Minnesota, Mississippi, Montana, North Dakota, Nebraska, New York, New Mexico, South Dakota, Utah, Virginia, Wisconsin, West Virginia, Iowa, Michigan, Missouri, Pennsylvania, Arkansas, Texas, and Wyoming.

Since 1997, CWD has been found in farmed cervids in 16 States: Colorado, Kansas, Michigan, Minnesota, Missouri, Montana, New York, Oklahoma, South Dakota, Iowa, Nebraska, Ohio, Pennsylvania, Texas, Utah and Wisconsin."

NOTE: There are 3 States (Oklahoma, Ohio, and Utah) with CWD only in captive herds. There are 8 States (Arkansas, Illinois, Maryland, Mississippi, New Mexico, West Virginia, Virginia, and Wyoming) with CWD only in wild populations.



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