RT-QUIC DETECTION OF CHRONIC WASTING DISEASE PRION IN ELISA-POSITIVE, IHC-NEGATIVE CERVID RPLN SAMPLES

Jenelle Catalina*, Keara Allen[1], Estela Kobashigawa[1], Shuping Zhang[1,2], Michael Zhang[1,2]

1: Veterinary Medical Diagnostic Laboratory, 2: Department of Veterinary Pathobiology,
College of Veterinary Medicine, University of Missouri-Columbia, Columbia, MO
*Questions on the poster can be sent to jcrp9@missouri.edu

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INTRODUCTION

- Chronic wasting disease (CWD) is a prion disease characterized by accumulation of abnormally folded isoform of the cellular prion protein (PrPC), named PrPSc, in the medulla oblongata (obex) region of the brain, as well as other tissues (lymph nodes, spleen, tonsils, etc.)
- CWD poses a significant risk to cervid populations, and some studies have raised concern for potential risk to humans.
- Enzyme-linked immunosorbent assay (ELISA) is routinely used for initial screening, followed by immunohistochemistry (IHC) for definitive diagnosis.
- RT-QuIC, a seeded amplification assay, is a potential alternative diagnostic assay that provides advantages over standard ELISA/IHC.

OBJECTIVES

- Evaluate the performance of the RT-QuIC assay in detecting CWD prion in archived, suspected positive (ELISA-positive, IHC-negative), retropharyngeal lymph node (RPLN) samples.
- Enhance our understanding of the comparative diagnostic accuracy of RT-QuIC relative to current methods, potentially informing future diagnostic practices.

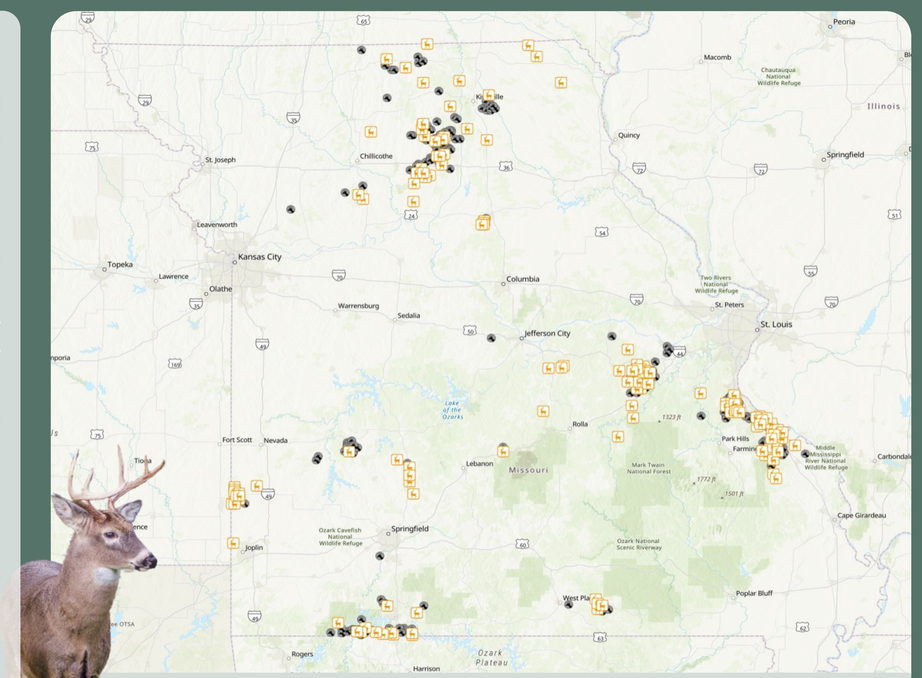

Figure 1. Map showing cases of CWD in free-ranging deer in Missouri. The Missouri Department of Conservation has seen a 9% increase in CWD cases since the department's 2022-2023 study.

METHODS

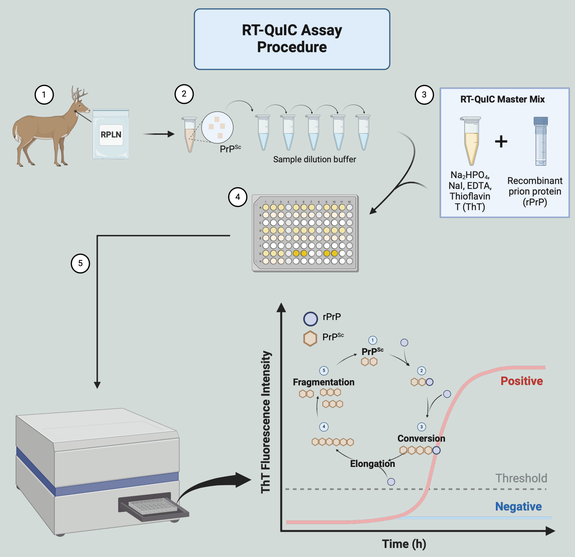

Figure 2. Schematic Representation of the RT-QuIC Assay for Detecting Prion Protein Aggregation. Step 1) Collection of RPLNs from white-tailed deer, Step 2) Serial dilutions of homogenized RPLNs, Step 3) Preparation of the RT-QuIC Master Mix, Step 4) Addition of serial diluted sample and reaction mixture to 96-well plate, Step 5) Incubation at 42°C for 72 hours with cycles of 1 min shaking, 1 min rest.

RESULTS

Table 1. Number of positive, negative, and total of RLNs tested by each assay

	Positive	Negative	N/A	Total
ELISA	122	0	0	122
IHC	0	122	0	122
RT-QuIC	3	43	76	46*

*Due to time constraints, RT-QuIC was performed on 46 out of 122 ELISA-positive and IHC-negative samples. N/A, data not available for these samples.

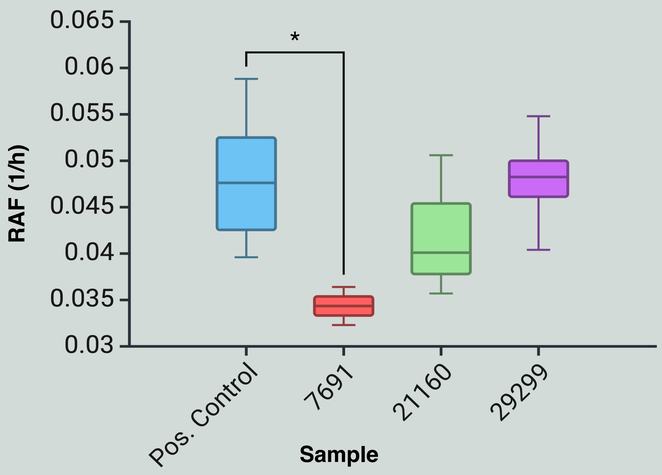

Figure 3. Detection of prion seeding activity in RT-QuIC CWD-positive RLNs. Rate of amyloid formation (1/h) was plotted using data collected from CWD ELISA-positive, IHC-negative, RT-QuIC positive samples. Statistical significance was obtained through comparing with rate of amyloid formation with positive controls used across the study (*p < 0.05).

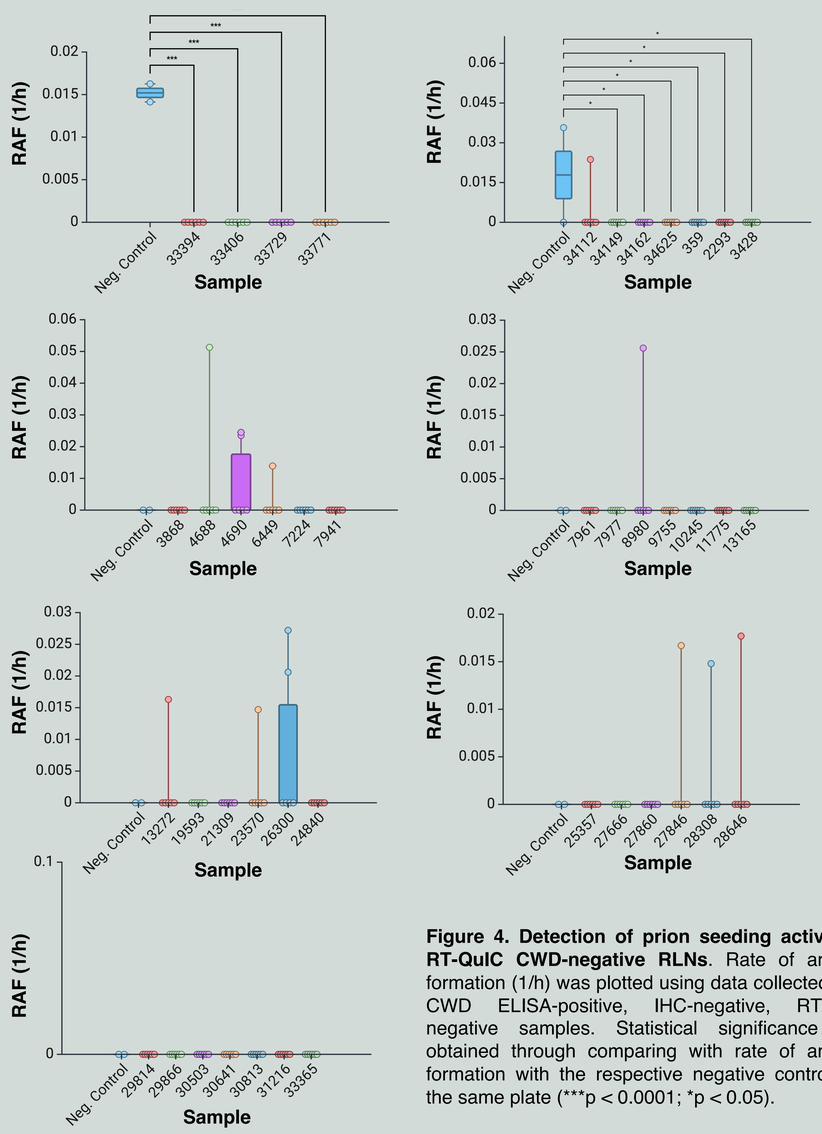

Figure 4. Detection of prion seeding activity in RT-QuIC CWD-negative RLNs. Rate of amyloid formation (1/h) was plotted using data collected from CWD ELISA-positive, IHC-negative, RT-QuIC negative samples. Statistical significance was obtained through comparing with rate of amyloid formation with the respective negative controls on the same plate (***p < 0.0001; *p < 0.05).

DISCUSSION

- Two samples exhibited mean RAF that were not significantly different from the positive control. This likely indicates true-positive CWD samples and it is recommended these samples undergo repeat IHC testing.
- There was no significant difference in RAF observed between the RT-QuIC-negative samples and negative controls from Run 3 through Run 7. This consistency in results indicates a reliable similarity between negative samples and the negative controls, reinforcing the validity of the RT-QuIC assay in these runs.
- Significant difference between negative samples and controls in Run 1 and Run 2 are attributed to spontaneous prion conversion and possible control contamination/procedural error, respectively.

CONCLUSION

- RT-QuIC demonstrated high concordance with established IHC methods, suggesting a high specificity of RT-QuIC in line with IHC results.
- Pending completion of RT-QuIC analysis on the 76 remaining ELISA+/IHC- samples, retesting of RT-QuIC Run 2, and additional IHC testing of samples 21160 and 29299, these findings support the utility of RT-QuIC as a reliable supplementary diagnostic tool for CWD

ACKNOWLEDGEMENTS

This research was supported by a grant from the **Missouri Department of Conservation** and scholar stipend from the **MU Veterinary Medical Diagnostic Laboratory**. We are grateful for their generous support. Tables and figures were created with **BioRender.**

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