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Sensitive detection of chronic wasting disease prions recovered from environmentally relevant surfaces

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Abstract

Chronic wasting disease (CWD) has been identified in 30 states in the United States, four provinces in Canada, and recently emerged in Scandinavia. The association of CWD prions with environmental materials such as soil, plants, and surfaces may enhance the persistence of CWD prion infectivity in the environment exacerbating disease transmission. Identifying and quantifying CWD prions in the environment is significant for prion monitoring and disease transmission control. A systematic method for CWD prion quantification from associated environmental materials, however, does not exist. In this study, we developed an innovative method for extracting prions from swabs and recovering CWD prions swabbed from different types of surfaces including glass, stainless steel, and wood. We found that samples dried on swabs were unfavorable for prion extraction, with the greatest prion recovery from wet swabs. Using this swabbing technique, the recovery of CWD prions dried to glass or stainless steel was approximately 30% in most cases, whereas that from wood was undetectable by conventional prion immunodetection techniques. Real-time quake-induced conversion (RT-QuIC) analysis of these same samples resulted in an

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CRediT authorship contribution statement

Qi Yuan: Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Gage Rowden:** Data curation, Writing – original draft, Writing – review & editing. **Tiffany M. Wolf:** Investigation, Writing – review & editing. **Marc D. Schwabenlander:** Investigation, Writing – review & editing. **Peter A. Larsen:** Resources, Investigation, Writing – review & editing. **Shannon L. Bartelt-Hunt:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing. **Jason C. Bartz:** Conceptualization, Funding acquisition, Investigation, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Qi Yuan, Tiffany M. Wolf, Marc D. Schwabenlander, Peter A. Larsen, and Jason C. Bartz have a patent application.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107347.

increase of the detection limit of CWD prions from stainless steel by 4 orders of magnitude. More importantly, the RT-QuIC detection of CWD prions recovered from stainless steel surfaces using this method was similar to the original CWD prion load applied to the surface. This combined surface swabbing and RT-QuIC detection method provides an ultrasensitive means for prion detection across many settings and applications.

Keywords

chronic wasting disease (CWD); Prion; Swab; Surface; Recovery; RT-QuIC

1. Introduction

Prion diseases impact a wide range of host species that include humans (Creutzfeldt-Jakob disease, CJD) (Matthews, 1978; Stengel and Wilson, 1946), cattle (bovine spongiform encephalopathy, BSE or “mad cow disease”) (Holt and Phillips, 1988), cervids (chronic wasting disease, CWD) (Williams and Young, 1980; Williams and Young, 1982), dromedary camels (Babelhadj et al., 2018) and sheep and goats (scrapie) (Greig, 1950; Hourriga and l., Klingspo, A. l., McDaniel, H. A., N., R. M., , 1969). Prions are comprised of PrP^{Sc}, a misfolded self-propagating form of normal cellular prion protein PrP^{C}. Compared to PrP^{C}, PrP^{Sc} has a higher content of β-sheet structure, is resistant to environmental degradation and to inactivation by methods used for conventional pathogens (Bellinger-Kawahara et al., 1987; Brown et al., 1990; Brown et al., 1982; Prusiner, 2004; Gibbs et al., 1978; Taylor and Diprose, 1996).

CWD is an emerging prion disease in North America and Europe. CWD has been detected in 30 states (xxxx) in the United States and four Canadian provinces (xxxx). The recent discovery of CWD in reindeer and moose in Norway, Finland, and Sweden (Benestad et al., 2016; Osterholm et al., 2019) has raised concerns about CWD transmission and emergence worldwide. In CWD endemic areas, the disease incidence in free-ranging deer herds can be>50% and can negatively impact cervid populations (Miller and Wild, 2004).

Prions that are released into the environment through animal excreta or carcass decomposition can be acquired by cervids from the environment (Miller and Williams, 2003; Miller et al., 2004). Prions can enter the environment from infected animals through excreta such as saliva, urine, and feces during both the asymptomatic and symptomatic phases of disease (Haley et al., 2011; Haley et al., 2009; Henderson et al., 2017; Gough and Maddison, 2010; Mathiason et al., 2009). CWD prions are also found in antler velvet and blood (Angers et al., 2009; Mathiason et al., 2006). At the terminal phase of disease, prions are found throughout the body of CWD-infected animals with the highest levels identified in the central nervous system (CNS) and lower amounts present in lymphoid tissue, fat, and muscle (Sigurdson, 2008; Race et al., 2009). The relative contribution of prions that enter the environment via excreta (relatively low prion load over a long period of time over a larger geographic area) versus prions that enter the environment from a carcass (relatively high prion load at one time point and location) are poorly understood.

Prion infectivity can persist in the environment for extended periods of time. Experimentally, prions buried in soil can survive under natural conditions for at least 3 years (Brown and Gajdusek, 1991; Seidel et al., 2007) while mouse-adapted BSE prions can survive burial for at least 5 years (Somerville et al., 2019). When incubated with soils, prions can retain seeding activity, a prion infectivity indicator, and/or infectivity after 1 to 3 years (Saunders et al., 2011; Kuznetsova et al., 2020) at the minimum. Prions are resistant to simulated weathering degradation, especially dehydrated on a surface (Yuan et al., 2015; Yuan et al., 2018). In an aqueous environment, prions can persist in sewage for up to eight years, and can survive simulated wastewater treatment with both activated and mesophilic anaerobic sludge digestion processes (Hinckley et al., 2008; Maluquer de Motes et al., 2012; Marin-Moreno et al., 2016). Overall, the resistance of prions to environmental degradation may contribute to environment-mediated disease transmission (Mathiason et al., 2009).

Prion-contaminated surfaces or plants may facilitate the environmental transmission of prions. Upon entry into the environment, prions can associate with soils and other environmental materials while retaining infectivity (Saunders et al., 2011; Saunders et al., 2009; Johnson et al., 2006). Prions can bind to whole soils and soil components, with clay and clayey soils having high sorption capacity and affinity in contrast to sand or sandy soils that have a lower capacity and affinity to bind prions (Saunders et al., 2009). Adsorption of prions to soils in a biological matrix, such as brain homogenate, has a lower adsorption capacity and rate compared to prions in less complicated matricies (Saunders et al., 2009). In addition to soil, prions can bind to other environmental surfaces such as plants, metals, plastic, rocks, and wood and can be taken up by plants from contaminated soils (Pritzkow et al., 2018; Pritzkow et al., 2015).

Environmental transmission of prions has been observed that is consistent with the long-term persistence of prions binding to surfaces in the environment. Introduction of sheep to pastures that once contained scrapie-infected sheep can result in the development of scrapie in newly introduced animals suggesting environmental transmission of scrapie (Georgsson et al., 2006). Removal of CWD-infected deer from paddocks two years prior to reintroduction of naive deer into the same paddocks resulted in the deer developing CWD (Miller et al., 2004). Consistent with these field observations, in confinement, environmental transmission of CWD in deer can occur (Mathiason et al., 2009) and transmission of prions from experimentally contaminated surfaces to rodents has been documented (Pritzkow et al., 2018).

The ability to detect environmentally-associated CWD prions is needed to effectively monitor and control this burgeoning disease. Currently, the only means for discovery of CWD infection in a new area and monitoring of CWD prions in CWD-endemic regions is by detection of prions in CWD-infected animals. The methodology for PrP^{Sc} detection in lymphoreticular system (LRS) or CNS tissues from cervids is robust and the infrastructure to support the diagnostic testing is widely available (McNulty et al., 2019; Schwabenlander et al., 2022). A downside of the current CWD testing regime is that samples must come from either captive, hunter-harvest, or from animals of opportunity (e.g., cervids killed by vehicles). This both temporally and spatially biases the sampling, therefore, systematic methods for determining if CWD-infected animals are present are needed. Since prions

are shed into the environment and it is known that environmental transmission can spread CWD infection, methods for detection of environmental CWD prions would complement the existing testing strategies. The use of surface sampling, in a captive facility or in a free-ranging location, may provide a practical method for prion recovery and detection. The objective of this study was to develop a sensitive method of CWD prion detection from environmentally relevant surfaces.

2. Materials and methods

2.1. Prion sources and tissue preparation

Brain tissues were collected from hamsters infected with the hyper (HY) strain of transmissible mink encephalopathy (TME) and from three elk infected with CWD designated as isolate CWD t1821, CWD JB R296, CWD JB B188, and CWD 307CL confirmed by Western blot. CWD t1821, CWD JB R296, and CWD JB B188 brains were homogenized to 10% (w/v) (20% for CWD t1821) in Dulbecco's phosphate-buffered saline (DPBS) without Ca^{2+} or Mg^{2+} (Mediatech, Herndon, VA) using strain-dedicated Tenbroeck tissue grinders (Kontes, Vineland, NJ). They were used in prion detection and recovery quantification from swabs and surfaces with 96-well immunoassay at Creighton. CWD 307CL, which had been successfully detected and characterized through real-time quaking-induced conversion (RT-QuIC) by Minnesota Center for Prion Research and Outreach (MNPRO), was homogenized to 10% (w/v) in PBS with Beadblaster 24 Microtube Homogenizer (D2400, Benchmark Scientific, Inc., Sayreville, NJ, US) and used in prion detection recovered from swabs and surfaces with RT-QuIC at University of Minnesota. Samples were stored at −80 °C.

2.2. Swab contamination

Cotton-tipped swabs (3M™ Quick Swab, 3 M, Saint Paul, MN, US) and foam-tipped swabs (Fisherbrand™ PurSwab Foam Swabs, Cat. No: 14–960-3E, Thermo Fisher Scientific, Waltham, MA, US) were used. To contaminate the swabs with prions for immunoassay detection, 500 (HY TME, CWD JB B188, and CWD JB R296) or 1000 (CWD t1821) μg brain equivalents (BE) of brain homogenate (BH) was applied to swabs by pipet. Contaminated swabs in quadruplicate were incubated at 22 °C to dry for 0 hr (undried control), 0.25 hr, 0.5 hr, 1 hr, 6 hrs, 12 hrs, or 24 hrs. After drying, swabs were immediately stored in 1.7 mL microcentrifuge tubes with 300 μL (for foam swabs) or 500 μL (for cotton swabs) of DPBS to completely cover the swab tips. To contaminate foam swabs for RT-QuIC detection, a 10-fold serial dilution of CWD 307CL from log −2 to −6 was prepared in DPBS. Triplicate aliquots of 50 μL from each dilution were applied to swabs by pipet. Swabs were immediately placed in 1.7 mL microcentrifuge tubes pre-loaded with 250 μL of DPBS. Handles of swabs were cut to fit in the tube with the cap closed.

2.3. Surface contamination and swabbing

Ten-fold serial dilutions of brain homogenates ranging from 500 to 0.5 (HY TME, CWD JB B188, and CWD JB R296) or 1000 to 1 (CWD t1821) μg BE were applied to positively charged glass slides (Fisherbrand Superfrost Plus Microscope slides, Catalog No.: 12–550-15), stainless steel sheets (316L grade, Millard Metal Services, La Vista, NE, US),

or oak wood coupons (Lowe's, Omaha, NE, US). Contaminated surfaces were dried at room temperature for 24 hrs then sampled with foam-tipped swabs. A swab was hydrated with ultrapure water then applied to the surface 10 times with spinning to maximize the exposure to the swab surface. To prepare positive controls, surfaces contaminated with the highest levels of prions (500 μg BE for CWD JB B188 and CWD JB R296; 1000 μg BE for CWD t1821) were sampled with dry swabs immediately following contamination, in a manner to absorb all liquid. Surfaces without prion-contamination (negative controls) were sampled as described for the prion-contaminated surfaces. To prepare prion-contaminated surface for RT-QuIC detection, 50 μL from each dilution of CWD 307CL (log −2 to −6) were applied to stainless steel and dried at 22 °C for 24 h followed by swabbing with foam-tipped swabs as described above. Surfaces were prepared in triplicate for each sample including positive and negative controls. One swab was used for each area as subsequent swabs could not recover detectable prions according to previous experiments (Table S1). Swab tips cut to fit the tubes were placed in 1.7 mL microcentrifuge tubes with 300 μL of DPBS in each to cover the foam tips and extracted immediately.

2.4. Swab extraction

Swabs in microcentrifuge tubes were either incubated with shaking or sonication for prion extraction. With shaking extraction, undried or dried swabs were incubated in 300 μL (for foam swabs) or 500 μL (for cotton swabs) of DPBS and agitated on a microtube mixer (Tomy MT-360, speed 5) at 22 °C for 30 min for the 1st extraction, followed with 2 serial extractions by incubating the swabs in a different microcentrifuge tube with 200 μL (for foam swabs) or 300 μL (for cotton swabs) of DPBS for 30 min for each extraction. With sonication extraction, swabs stored in microcentrifuge tubes were placed in a QSonica sonicator (Q700) with a microplate horn (Part #: 431MPX). The amplitude was set to level 17, generating an average output of around 175 W during sonication treatment, with an approximate 1.25 Watts/cm^2 ultrasonic intensity. Depending on the desired length of total treatment time for each extraction (5 s, 15 s, 30 s, or 60 s), sonication was performed at 37 °C and consisted of different numbers (1, 3, 6, or 12) of treatment cycles (5 s of sonication followed by 5 s of incubation). For each swab, three serial extractions with the same length of sonication time were applied. The buffer usage for sonication extraction was also the same as for shaking. Extracts (~200 μL each for foam swabs, ~ 300 μL each for cotton swabs) were stored at −80 °C until ready for concentration. Foam swabs for CWD 307CL for RT-QuIC analysis were extracted using sonication as described above. Two serial extractions were conducted for each swab.

Swabs used for surface sampling were extracted twice (one time for swabs of negative surface controls for immunodetection) using 15 s of sonication for each extraction (3 treatment cycles). Extracts were either combined (~400 μL for swabs for immunodetection) or stored separately (~200 μL for swabs for RT-QuIC). All extracts were stored at −80 °C.

2.5. Extract concentration

All extracts for the immunodetection were vacuum concentrated with Savant Speed-Vac concentrator equipped with Savant refrigerated vapor trap (RVT4104). Samples were evaporated (65 °C in the chamber) for 3 hrs, 4 hrs, or 5 hrs for 200 μL, 300 μL, or 400 μL of

extracts, respectively. Concentrated extracts were rehydrated with 10 μL, 15 μL, or 20 μL of ultrapure water correspondingly. Extracts for RT-QuIC detection were vacuum concentrated with SpeedVac (SPD 1030, Thermo Fisher Scientific, Waltham, MA, US). Samples were evaporated at 45 °C for 2 h with 10 Torr vacuum and rehydrated with 50 μL of ultrapure water. Concentrated extracts were stored at −80 °C before analysis.

2.6. 96-well immunodetection and recovery quantification

Samples were digested with 23.25 μg/ml (HY TME, CWD JB B188, and CWD JB R296) or 46.5 μg/ml (CWD t1821) proteinase K (PK) (Roche Diagnostics Corporation, Indianapolis, IN) at 37 °C for 30 min with constant agitation. 96-well immunoblot assay was performed as described previously (Kramer and Bartz, 2009) with primary monoclonal antibody 3F4 (Sigma-Aldrich, St. Louis, MO, US; 0.1 μg/mL, 37 °C for 1 hr) for HY TME or 8H4 (Sigma-Aldrich, St. Louis, MO, US; 0.17 μg/mL, 37 °C for 1 hr) for CWD and secondary antibody (horseradish-peroxidase conjugated antimouse IgG, Invitrogen, Carlsbad, CA, US; 0.01 μg/mL, 37 °C for 30 min). Well-plate membranes were developed with Supersignal West Femto maximum sensitivity substrate, according to the manufacturer's instructions (Pierce, Rockford, IL, US), imaged on a 4000R imaging station (Kodak, Rochester, NY), and analyzed using Kodak (New Haven, CT) molecular imaging software (V.5.0.1.27), which output the net intensity of each well. For each plate, 2-fold dilutions of the control were prepared to generate a linear regression of PK-resistant prion protein (PrP^{Res}) intensity (standard curve). Blank wells with DPBS only and negative control in triplicates with healthy uninfected hamster brain homogenate were tested in each plate. Incomplete PK digestion of uninfected hamster brain was not observed. An aliquot of recovered PrP^{Res} from swabs or surfaces (e.g., 1/10 in order to generate a signal within the linear range) was loaded to the wells and calculated for PrP^{Res} amount according to the standard curve. The total amount of recovered PrP^{Res} were then adjusted for aliquoting. The unit of PrP^{Res} used in this study was μg brain equivalent (BE) referring to PrP^{Res} content in corresponding μg of wet weight of the brain tissue. The recovery was determined as the ratio of total amount of recovered PrP^{Res} to the amount of PrP^{Res} originally loaded. Statistical analysis was performed with unpaired parametric Welch's *t-test* using GraphPad Prism 8 (GraphPad Software, Inc., CA, US).

2.7. RT-QuIC reaction

RT-QuIC was performed and analyzed as previously described (Schwabenlander et al., 2022; Wilham and Orrú, C. D., Bessen, R. A., Atarashi, R., Sano, K., Race, B., Meade-White, K. D., Taubner, L. M., Timmes, A., Caughey, B., , 2010). Briefly, control serial dilutions and swab extracts from above were diluted 10-fold in 0.1% SDS/1X PBS/1x N2. 2 μL of control and swab extract diluent were added to 98 uL of RT-QuIC reaction buffer. The reaction buffer was made to the following concentrations: 1X PBS, 170 mM NaCl, 1 mM EDTA, 10 μM thioflavin T (ThT), and 0.1 mg/mL recombinant hamster prion protein (recHaPrP). The reactions were read for ThT fluorescence which emits light with maximum excitation/emission at approximately 450/480 every 45 min for 48 h. Shaking was performed at 700 rpm double orbital for 1 min and rest for 1 min. A maxpoint ratio (MPR) was calculated by taking the maximum fluorescence of each well and dividing it by the initial fluorescence (i.e., the background fluorescence) (Vendramelli et al., 2018). A rate of amyloid formation

(RAF) was also calculated per well as the reciprocal of the time necessary for fluorescence to reach twice the background fluorescence.

3. Results

3.1. Surface drying decreased prion recovery from swabs extracted under gentle shaking

The detection of recovered HY PrP^{Res} (Fig. 1. A, B, C, D) and CWD t1821 PrP^{Res} (Fig. 1. E, F, G, H) and recovery quantification from each extraction (individual recovery) and 3 sequential extractions (total recovery) of the swabs dried for different times are presented. Total recovery of HY PrP^{Res} from the foam swab without drying (0 hr) was 49% ± 1% (mean ± standard error of mean) (Fig 1. B). After 15 min of drying (0.25 hr), total HY PrP^{Res} recovery from the foam swab maintained similarly ($p > 0.05$) at 50% ± 4% and significantly decreased ($p < 0.05$) to 36% ± 2% after 30 min of drying (0.5 hr) (Fig. 1. B). After 1 hr of drying, total extracted HY PrP^{Res} was near the limit of PrP^{res} detection (Fig. 1. A) and the total recovery reduced significantly ($p < 0.05$) to approximately 1% (Fig. 1. B). Application of HY to cotton swabs (Fig. 1. C, D) resulted in 51% ± 12% total recovery of PrP^{Res} without drying (0 hr) that after 15 min of drying (0.25 hr) was significantly decreased ($p < 0.05$) to<6%. Similar results were observed for CWD. From foam swabs, total recoveries of CWD PrP^{Res} after 0hr, 0.25 hr, and 0.5 hr of drying were 68% ± 7%, 58% ± 3%, and 59% ± 7%, respectively (Fig. 1. E, F). After 1 hr of drying, total extracted CWD PrP^{Res} significantly decreased ($p < 0.05$) to<5% (Fig. 1. E, F). From cotton swabs, 43% ± 3% of CWD PrP^{Res} was recovered without drying (0 hr) and the total CWD recoveries thereafter significantly reduced ($p < 0.05$) to 12% or lower (Fig. 1. G, H).

To investigate if the difference between foam swabs and cotton swabs is related to drying dynamics, the moisture content, calculated as a percentage of liquid weight after drying to initial loading weight, of both types of swabs containing either HY or CWD PrP^{res} or the same volume of ultra-purified water were determined. The overall trends of swab moisture content are similar among all conditions, with a moisture content reduction to approximately 10% after 30 min of surface drying and a further reduction through 1 hr of drying (Fig. 1), suggesting that differences in moisture content between the cotton and foam swabs did not affect prion recovery.

3.2. Enhancement of HY recovery from dried foam swabs using sonication

To investigate whether a more energetic mechanical force can improve the extraction efficiency for surface-dried PrP^{res}, sonication was used to replace the gentle shaking. Sonication of foam swabs for 5 s, 15 s, 30 s, and 60 s immediately after application of HY (Fig. 2. A, B), resulted in total HY PrP^{Res} recoveries of 66% ± 16%, 58% ± 4%, 51% ± 4%, and 52% ± 5%, respectively. Recoveries of HY PrP^{Res} were similar ($p > 0.05$) between the tested sonication durations and that from undried foam swabs extracted with gentle shaking (Fig. 1. A, B). After 24 h of drying on foam swabs, total HY PrP^{Res} recoveries were 22% ± 6%, 46% ± 14%, 57% ± 21%, and 41% ± 18% for the tested sonication durations from short (5 sec) to long (60 sec). The recovery of PrP^{Res} from the sonication-treated dried foam swabs was generally significantly ($p < 0.05$) greater than from dried swabs (≥1 hr) extracted

with gentle shaking (Fig. 1. A, B). Overall, sonication increased the recovery of PrP^{Res} from dried foam swabs.

3.3. Recovery of surface-dried CWD from foam swabs by sonication extraction varied among isolates

To examine the efficiency of sonication extraction for surface-dried CWD from foam swabs, CWD contaminated swabs were either extracted immediately (0 hr) or after 24 h of drying (24 hr) with 15 s of sonication. After three sequential extractions, total CWD PrP^{Res} recoveries from undried foam swabs were 57% ± 4% for CWD t1821, 50% ± 5% for CWD JB R296, and 34% ± 3% for CWD JB B188 (Fig. 3). After 24-hour drying, total CWD PrP^{Res} recoveries decreased significantly ($p < 0.05$) to 13% ± 2%, 32% ± 3%, and 10% ± 2%, respectively. Total recovery of PrP^{Res} from the undried foam swabs contaminated with either CWD t1821 or CWD JB R296 were significantly ($p < 0.05$) higher compared to foam swabs contaminated with CWD JB B188, whereas from 24-hr dried foam swab, total recovery of CWD JB R296 was higher ($p < 0.05$) than the other samples. Recovery of CWD PrP^{Res} from dried swabs was enhanced with sonication extraction. From undried foam swabs, total CWD t1821 recovery was similar ($p > 0.05$) between with gentle shaking and with sonication (Fig. 1. E, F and Fig. 3. A, B). Sonication extraction from dried foam swabs resulted in greater ($p < 0.05$) CWD PrP^{Res} recovery compared to shaking extraction (Fig. 1. E, F and Fig. 3. A, B).

3.4. Prion recovery from glass and stainless steel was higher than from wood

Prions from three environmentally relevant surfaces contaminated with three CWD isolates were sampled using foam swabs. PrP^{Res} was extracted from the swab using short sonication extractions (15 s) (Fig. 4). Due to unknown effect of surface drying to prion recovery, controls with immediate swabbing after surface contamination with the highest level of CWD prions (1000 μg BE for CWD t1821, 500 μg BE for CWD JB R296 and CWD JB B188) and immediate swab extraction were prepared. Recoveries of CWD PrP^{Res} from control surfaces ranged from 25% to 99% for glass and stainless steel surfaces, with the majority around 30% (Table 1 and Fig. 4. A-I). In contrast, contamination of wood with CWD resulted in significantly lower ($p < 0.05$, except for Figure G vs. J and I vs. L) PrP^{Res} recoveries for all CWD isolates tested (11% ~ 16%) from undried surface (control) (Table 1 and Fig. 4. A-C, J-L). At the highest contamination levels, all CWD isolates had similar ($p > 0.05$) recovery from glass and stainless steel after 24 h of drying on surface to their controls (Table 1 and Fig. 4. A-I). However, extracted CWD PrP^{Res}, at the highest contamination levels, from wood after 24 h of drying were significantly reduced ($p < 0.05$) compared to their controls (Table 1 and Fig. 4. A-C, J-L). At all other lower contamination levels, CWD PrP^{Res} was not detected regardless of the surface type. Swab extracts from surfaces without prion contamination (negative controls) generated undetectable CWD PrP^{Res} (Table S2) for all surface types.

3.5. RT-QuIC detection of CWD prion from swabs and stainless steel surface

To apply the optimized prion swab extraction method to the detection of prions swabbed from surfaces the following experimental method was employed. First, serial 10-fold dilutions of CWD-infected brain (isolate 307CL) were prepared ranging from 10^{-2} (10

μg brain equivalents/ml) to 10^{-6} (0.001 μg brain equivalents/ml) (Fig. 5A). These dilutions were directly analyzed by RT-QuIC resulting in a detection limit of 10^{-5} (Fig. 5B/5C controls). Next the same serial 10-fold dilutions of CWD-infected brain were applied to foam-tipped swabs, extracted using the optimized protocol and analysis of the extracts using RT-QuIC resulted in detection limit of 10^{-5} in all three experimental replicates (Fig. 5A/5B). Finally, the same serial 10-fold dilutions of CWD-infected brain were applied to stainless steel surface, dried for 24 h at 22 °C, then the surfaces were swabbed, the swabs were extracted using the optimized protocol and analysis of the extracts using RT-QuIC resulted in detection limit of 10^{-5} in all three experimental replicates (Fig. 5A/5C). The RT-QuIC CWD detection limit was similar between the direct analysis of the serial 10-fold dilutions of CWD-infected brain, the analysis of CWD directly applied to swabs (Fig. 5B pooled) and CWD applied to a stainless steel surface, dried for 24 h and then swabbed (Fig. 5C pooled). CWD was extracted a 2nd time from the same swabs to determine if additional RT-QuIC seeding activity could be recovered. The RT-QuIC seeding activity from a 2nd extraction of CWD prions directly applied to the swabs (Fig. 5B) or recovered from surface (Fig. 5C) was decreased by 1 log compared to the first extraction (Figure S1). Consistent results were observed for both the 1st (Figure S2) and 2nd (Figure S3) extracts in the RAF analysis. The extracts of negative controls which were DPBS-loaded swabs and uncontaminated surfaces did not initiate RT-QuIC reactions (Figure S4). Overall, RT-QuIC detection of CWD prion recovered from stainless steel surface by swab sampling was comparable to detection of CWD prion when directly added to the RT-QuIC reactions.

4. Discussion

The development of methods for directly measuring prion infectivity in environmental samples is needed to help control CWD. Conventional immunoassays such as Western blot have failed to detect PrP^{Sc} in environmental samples (Maddison et al., 2010; Nichols et al., 2009). This may be due to levels of PrP^{Sc} in the environmental samples that are below the limit of detection of these assays. Additionally, recovery of PrP^{Sc} from environmental samples prior to analysis may be inefficient. Here we show a novel method of surface swabbing and extraction where we achieve up to 30% prion recovery. This may be an underrepresentation, however, since our technique included a PK digestion step with a constant conentration of PK that would eliminate sensitive forms of PrP^{Sc} and may lead over digestion of prions in the second or third extracts. While complete recovery of PrP^{Sc} from both types of swabs was not observed for either HY or CWD prions, we identified critical parameters that increased the extraction yield from the swabs that were compatible with sensitive detection methods.

Drying on the swabs reduced prion recovery. While immediate extraction following prion contamination recovered the majority of Prp^{Res} from swabs, the amount of recovered PrP^{Res} decreased after drying (Figs. 1, 3). The impact of drying on prion recovery over time corresponded with the swab materials and not with the prion strain. Recovery of PrP^{Res} from foam-tipped swabs made of polyurethane structured with pores were less impacted by drying than cotton swabs tipped with densely compacted cellulose fibers (Fig. 1). Importantly, extended drying reduced CWD prion recovery which was not fully restored by increasing mechanical energy to desorb PrP^{Res} from the surface (Fig. 3). This finding

is consistent with previous studies where drying of prions to stainless steel decreases the effectiveness of prion decontamination compared to prions in a hydrated state (Secker et al., 2011). Variation of the CWD prion recovery was observed between isolates and differences in clinical stage when the animal died, PrP genotype, or prion strain could contribute to the observed variation. Overall, storing swabs in the extraction buffer after sampling is recommended for improved prion recovery. If dried swabs are used, however, we found that sonication can increase the recovery of PrP^{Res}. This may be due to either the physical separation of PrP^{Res} from the surface, and/or fragmentation of PrP^{Res}. The disassociation process to fragment PrP^{Res} from surface is thought to occur in protein misfolding cyclic amplification (PMCA) which recapitulates prion self-propagating process *in vivo* utilizing sonication (Saborio et al., 2001).

Recovery of prions was surface dependent. Recoveries of CWD PrP^{Sc} from glass and stainless steel surfaces were generally greater than from wood (Fig. 4). We hypothesize that the porosity of the wood surface allows liquids containing prions to enter the pore structure immediately after contact, leaving limited material for transferring to the swabs even without drying. Drying on the wood surface might further allow CWD prion penetration into the wood resulting in a failure to detect swab-sampled CWD PrP^{Sc} by 96-well immunoassay. Importantly, drying at least for 24 h did not impact the recoveries of CWD PrP^{Sc} from the glass or stainless steel surface as the recoveries from undried surface controls (containing the highest CWD PrP^{Sc} amount) were similar to those from the 24-hr dried surfaces with the highest contamination level (Fig. 4). This observation suggests that detection of prions from swab-sampled surfaces made of similar materials (e.g., metal fence material, contaminated equipment) may allow for more sensitive detection of CWD prions in natural settings. Overall, these findings suggest that for optimal sampling of prions in the environment, choice of surfaces will have a significant impact on prion recovery. It will be useful for design of environmental sampling stations that are placed in CWD endemic areas and captive facilities to aid in the monitoring of CWD or assessment of equipment decontamination.

The combination of the newly developed swab extraction method, the identification of a surface with higher prion recovery, and detection of PrP^{Sc} using RT-QuIC allowed for sensitive detection of environmental CWD prions. We found that the RT-QuIC detection sensitivity for CWD swabbed from stainless steel surface did not differ from the CWD prions directly added to the RT-QuIC reactions (Fig. 5), indicating that the PrP^{Sc} recovery of 25% ~ 65% from stainless steel (Fig. 4, Table 1) is sufficient for efficient RT-QuIC detection. Overall, we have developed an innovative method of recovery of prions from environmentally-relevant surfaces that, used in combination with ultrasensitive methods may provide a means for detection and quantification of prions from contaminated environmental surfaces or equipment.

5. Conclusions

Chronic wasting disease is spreading in North America and it is hypothesized that in CWD-endemic areas environmental persistence of CWD prions can exacerbate disease transmission. The development of a sensitive CWD prion detection method from

environmentally relevant surfaces is significant for monitoring, risk assessment, and control of CWD. In this study, we developed a novel swab-extraction procedure for field deployable sampling of CWD prions from stainless steel, glass, and wood. We found that extended swab-drying was unfavorable for extraction, indicating that hydrated storage of swabs after sampling aided in prion recovery. Recoverable CWD prions from stainless steel and glass was approximately 30%, which was greater than from wood. RT-QuIC analysis of the swab extracts resulted in an increase of the detection limit of CWD prions from stainless steel by 4 orders of magnitude compared to conventional immunodetection techniques. More importantly, the RT-QuIC detection of CWD prions recovered from stainless steel surfaces using this developed method was similar to the original CWD prion load without surface contact. This method of prion sampling and recovery, in combination with ultrasensitive detection methods, allows for prion detection from contaminated environmental surfaces.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Matthews WB, 1978. Creutzfeldt-Jakob disease. Postgrad. Med. J 54 (635), 591–594. [PubMed: 103082]

Stengel E, Wilson WEJ, 1946. Jakob-Creutzfeldt disease. Journal of Mental Science 92 (387), 370–378. [PubMed: 20990313]

Holt TA, Phillips J, 1988. Bovine spongiform encephalopathy. Br. Med. J 296 (6636), 1581–1582. [PubMed: 3135018]

Williams ES, Young S, 1980. Chronic wasting disease of captive mule deer - spongiform encephalopathy. J. Wildl. Dis 16 (1), 89–98. [PubMed: 7373730]

Williams ES, Young STUART, 1982. Spongiform encephalopathy of Rocky-Mountain elk. J. Wildl. Dis 18 (4), 465–471. [PubMed: 7154220]

Babelhadj B, Di Bari M, Pirisinu L, Chiappini B, Gaouar S, Riccardi G, Marcon S, Agrimi U, Nonno R, Vaccari G, 2018. Prion disease in dromedary camels. Algeria. Emerg. Infect Dis 24 (6), 1029–1036. [PubMed: 29652245]

Greig JR, 1950. Scrapie in sheep. Journal of Comparative Pathology and Therapeutics 60 (4), 263–266.

Hourriga J. l.; Klingspo A. l.; McDaniel HA; N. RM, Natural scrapie in a goat. Journal of the American Veterinary Medical Association 1969, 154, (5), 538-&. [PubMed: 5812892]

Bellinger-Kawahara C, Cleaver JE, Diener TO, Prusiner SB, 1987. Purified scrapie prions resist inactivation by UV irradiation. J. Virol 61 (1), 159–166. [PubMed: 3097336]

Brown P, Liberski PP, Wolff A, Gajdusek DC, 1990. Resistance of scrapie infectivity to steam autoclaving after formaldehyde fixation and limited survival after ashing at 360 degrees C: practical and theoretical implications. J. Infect. Dis 161, 467–472. [PubMed: 2107265]

Brown P, Rohwer RG, Green EM, Gajdusek DC, 1982. Effect of chemicals, heat, and histopathological processing on high infectivity hamster-adapted scrapie virus. J. Infect. Dis 145, 683–687. [PubMed: 6804575]

Prusiner SB, An introduction to prion biology and diseases. In Prion Biology and Diseases., 2nd ed.; Prusiner SB, Ed. Cold Spring Harbor: NY, 2004; pp 1–88.

Gibbs CJ, Gajdusek DC, Latarjet R, 1978. Unusual resistance to ionizing-radiation of the viruses of Kuru, Creutzfeldt-Jakob disease, and scrapie. PNAS 75 (12), 6268–6270. [PubMed: 104301]

Taylor DM, Diprose MF, 1996. The response of the 22A strain of scrapie agent to microwave irradiation compared with boiling. Neuropathol. Appl. Neurobiol 22 (3), 256–258. [PubMed: 8804028]

https://www.usgs.gov/centers/nwhc/science/expanding-distribution-chronic-wasting-disease?qt-science_center_objects=0#qt-science_center_objects (Accessed on April 20).

https://www.inspection.gc.ca/animal-health/terrestrial-animals/diseases/reportable/cwd/herds-infected/eng/1554298564449/1554298564710 (Accessed on April 21).

Benestad SL, Mitchell G, Simmons M, Ytrehus B, Vikoren T, 2016. First case of chronic wasting disease in Europe in a Norwegian free-ranging reindeer. Vet. Res 47, 88. [PubMed: 27641251]

Osterholm MT, Anderson CJ, Zabel MD, Scheftel JM, Moore KA, Appleby BS, 2019. Chronic wasting disease in cervids: implications for prion transmission to humans and other animal species. mBio 10 (4), e01091–e01119. [PubMed: 31337719]

Miller MW, Wild MA, 2004. Epidemiology of chronic wasting disease in captive white-tailedand mule deer. J. Wildl. Dis 40 (2), 320–327. [PubMed: 15362835]

Miller MW, Williams ES, 2003. Horizontal prion transmission in mule deer. Nature 425, 35–36. [PubMed: 12955129]

Miller MW, Williams ES, Hobbs NT, Wolfe LL, 2004. Environmental source of prion transmission in mule deer. Emerg. Infect. Dis 10, 1003–1006. [PubMed: 15207049]

Haley NJ, Mathiason CK, Carver S, Zabel MD, Telling GC, Hoover EA, 2011. Detection of chronic wasting disease prions in salivary, urinary, and intestinal tissues of deer: potential mechanisms of prion shedding and transmission. J. Virol 85 (13), 6309–6318. [PubMed: 21525361]

Haley NJ, Mathiason CK, Zabel MD, Telling GC, Hoover EA, Ma J, 2009. Detection of sub-clinical CWD infection in conventional test-negative deer long after oral exposure to urine and feces from CWD+ deer. PLoS ONE 4 (11), e7990. [PubMed: 19956732]

Henderson DM, Tennant JM, Haley NJ, Denkers ND, Mathiason CK, Hoover EA, 2017. Detection of chronic wasting disease prion seeding activity in deer and elk feces by real-time quaking-induced conversion. J. Gen. Virol 98 (7), 1953–1962. [PubMed: 28703697]

Gough KC, Maddison BC, 2010. Prion transmission: prion excretion and occurrence in the environment. Prion 4, 275–282. [PubMed: 20948292]

Mathiason CK, Hays SA, Powers J, Hayes-Klug J, Langenberg J, Dahmes SJ, Osborn DA, Miller KV, Warren RJ, Mason GL, Hoover EA, Westermark P, 2009. Infectious prions in pre-clinical deer and transmission of chronic wasting disease solely by environmental exposure. PLoS ONE 4 (6), e5916. 10.1371/journal.pone.000591610.1371/journal.pone.0005916.g00110.1371/journal.pone.0005916.g00210.1371/journal.pone.0005916.g00310.1371/journal.pone.0005916.g00410.1371/journal.pone.0005916.t00110.1371/journal.pone.0005916.t002. [PubMed: 19529769]

Angers RC, Seward TS, Napier D, Green M, Hoover E, Spraker T, O'Rourke K, Balachandran A, Telling GC, 2009. Chronic Wasting Disease Prions in Elk Antler Velvet. Emerg. Infect. Dis 15 (5), 696–703. [PubMed: 19402954]

Mathiason CK, Powers JG, Dahmes SJ, Osborn DA, Miller KV, Warren RJ, Mason GL, Hays SA, Hayes-Klug J, Seelig DM, Wild MA, Wolfe LL, Spraker TR, Miller MW, Sigurdson CJ, Telling GC, Hoover EA, 2006. Infectious prions in the saliva and blood of deer with chronic wasting disease. Science 314, 133–135. [PubMed: 17023660]

Sigurdson CJ, 2008. A prion disease of cervids: chronic wasting disease. Vet. Res 39, 41. [PubMed: 18381058]

Race B, Meade-White K, Race R, Chesebro B, 2009. Prion infectivity in fat of deer with chronic wasting disease. J. Virol 83, 9608–9610. [PubMed: 19570855]

Brown P, Gajdusek DC, 1991. Survival of scrapie virus after 3 year's interment. Lancet 337 (8736), 269–270. [PubMed: 1671114]

Seidel B, Thomzig A, Buschmann A, Groschup MH, Peters R, Beekes M, Terytze K, Khoury JE, 2007. Scrapie agent (strain 263K) can transmit disease via the oral route after persistence in soil over years. PLoS ONE 2 (5), e435. 10.1371/journal.pone.000043510.1371/journal.pone.0000435.g00110.1371/journal.pone.0000435.g00210.1371/journal.pone.0000435.g00310.1371/journal.pone.0000435.g00410.1371/journal.pone.0000435.t00110.1371/journal.pone.0000435.t002. [PubMed: 17502917]

Somerville RA, Fernie K, Smith A, Bishop K, Maddison BC, Gough KC, Hunter N, 2019. BSE infectivity survives burial for five years with only limited spread. Arch. Virol 164, 1135–1145. [PubMed: 30799509]

Saunders SE, Shikiya RA, Langenfeld K, Bartelt-Hunt SL, Bartz JC, 2011. Replication efficiency of soil-bound prions varies with soil type. J. Virol 85, 5476–5482. [PubMed: 21430062]

Kuznetsova A, McKenzie D, Cullingham C, Aiken JM, 2020. Long-term incubation prp^{CWD} with soils affects prion recovery but not infectivity. Pathogens 9 (4), 311. 10.3390/pathogens9040311. [PubMed: 32340296]

Yuan Q, Eckland T, Telling GC, Bartz JC, Bartelt-Hunt SL, 2015. Mitigation of prion infectivity and conversion capacity by a simulated natural process–repeated cycles of drying and wetting. Plos Pathog. 11, (2), e1004638. [PubMed: 25665187]

Yuan Q, Telling GC, Bartelt-Hunt SL, Bartz JC, 2018. Dehydration of prions on environmentally relevant surfaces protects them from inactivation by freezing and thawing. J. Virol 92 (8), e02191–e02217. [PubMed: 29386284]

Hinckley GT, Johnson CJ, Jacobson KT, Bartholomay C, McMahon KD, McKenzie DI, Aiken JM, Pedersen JA, 2008. Persistence of pathogenic prion protein during simulated wastewater treatment processes. Environ. Sci. Technol 42, 5254–5259. [PubMed: 18754377]

Maluquer de Motes C, Espinosa JC, Esteban A, Calvo M, Girones R, Torres JM, 2012. Persistence of the bovine spongiform encephalopathy infectious agent in sewage. Environ. Res 117, 1–7. [PubMed: 22776326]

Marin-Moreno A, Espinosa JC, Fernandez-Borges N, Piquer J, Girones R, Andreoletti O, Torres JM, 2016. An assessment of the long-term persistence of prion infectivity in aquatic environments. Environ. Res 151, 587–594. [PubMed: 27591838]

Saunders SE, Bartz JC, Bartelt-Hunt SL, 2009. Prion protein adsorption to soil in a competitive matrix is slow and reduced. Environ. Sci. Technol 43, 7728–7733. [PubMed: 19921886]

Johnson CJ, Phillips KE, Schramm PT, McKenzie D, Aiken JM, Pedersen JA, 2006. Prions adhere to soil minerals and remain infectious. Plos Pathog. 2, e32. [PubMed: 16617377]

Pritzkow S, Morales R, Lyon A, Concha-Marambio L, Urayama A, Soto C, 2018. Efficient prion disease transmission through common environmental materials. J. Biol. Chem 293 (9), 3363–3373. [PubMed: 29330304]

Pritzkow S, Morales R, Moda F, Khan U, Telling GC, Hoover E, Soto C, 2015. Grass plants bind, retain, uptake, and transport infectious prions. Cell Rep. 11, 1168–1175. [PubMed: 25981035]

Georgsson G, Sigurdarson S, Brown P, 2006. Infectious agent of sheep may persist in the environment for at least 16 years. J. Gen. Virol 87, 3737–3740. [PubMed: 17098992]

McNulty E, Nalls AV, Mellentine S, Hughes E, Pulscher L, Hoover EA, Mathiason CK, Caughey B, 2019. Comparison of conventional, amplification and bio-assay detection methods for a chronic wasting disease inoculum pool. PLoS ONE 14 (5), e0216621. [PubMed: 31071138]

Schwabenlander MD, Rowden GR, Li M, LaSharr K, Hildebrand EC, Stone S, Seelig DM, Jennelle CS, Cornicelli L, Wolf TM, Carstensen M, Larsen PA, 2022. Methods and procedures: implications for free-ranging white-tailed deer (*odocoileus virginianus*) surveillance and management. J. Wildl. Dis 58 (1), 50–62. [PubMed: 34695201]

Kramer ML, Bartz JC, 2009. Rapid, high-throughput detection of PrP^{Sc} by 96-well immunoassay. Prion 3 (1), 44–48. [PubMed: 19372734]

Wilham JM; Orrú CD; Bessen RA; Atarashi R; Sano K; Race B; Meade-White KD; Taubner LM; Timmes A; Caughey B, Rapid end-point quantitation of prion seeding activity with sensitivity comparable to bioassays. PloS Pathog. 2010, 6, (12), e1001217. [PubMed: 21152012]

Vendramelli R, Sloan A, Simon SLR, Godal D, Cheng K, 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). J. Clin. Microbiol 56, e00423–e00518. [PubMed: 29695523]

Maddison BC, Baker CA, Terry LA, Bellworthy SJ, Thorne L, Rees HC, Gough KC, 2010. Environmental sources of scrapie prions. J. Virol 84, 11560–11562. [PubMed: 20739536]

Nichols TA, Pulford B, Wyckoff AC, Meyerett C, Michel B, Gertig K, Hoover EA, Jewell JE, Telling GC, Zabel MD, 2009. Detection of protease-resistant cervid prion protein in water from a CWD-endemic area. Prion 3, 171–183. [PubMed: 19823039]

Secker TJ, Hervé R, Keevil CW, 2011. Adsorption of prion and tissue proteins to surgical stainless steel surfaces and the efficacy of decontamination following dry and wet storage conditions. J. Hosp. Infect 78 (4), 251–255. [PubMed: 21658801]

Saborio GP, Permanne B, Soto C, 2001. Sensitive detection of pathological prion protein by cyclic amplification of protein misfolding. Nature 411, 810–813. [PubMed: 11459061]

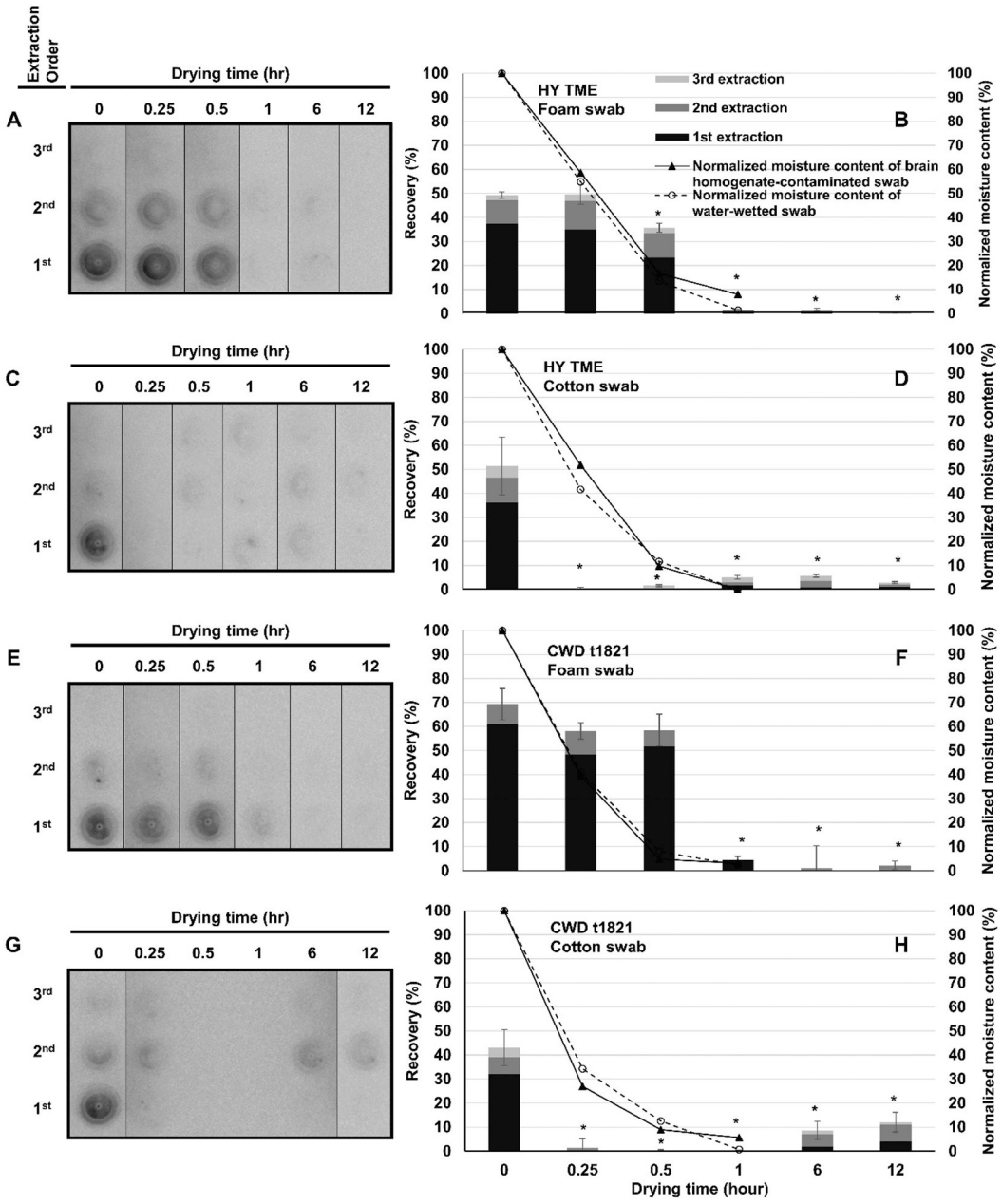

Fig. 1. Recovery of HY TME PrP^{res} from foam or cotton swabs using shaking.

Representative 96-well immunoblots (A, C, E, G) of recovered HY PrP^{Res} and recovery quantification (B, D, F, H column graph corresponding to A, C, E, G, respectively) from three sequential extractions (1st, 2nd, 3rd) of both foam and cotton swabs dried at room temperature for different lengths of time. Line graphs represent the moisture content of swabs wetted with HY TME brain homogenate (solid line) or ultrapure water (dashed line) after drying at room temperature. Results of PrP^{Res} recovery are expressed as mean of total recovery ± SEM, n = 4; data points in the line graphs represent the mean value of moisture content, n = 3. * indicates significant difference ($P < 0.05$) between the sample and respective undried control (0 hr).

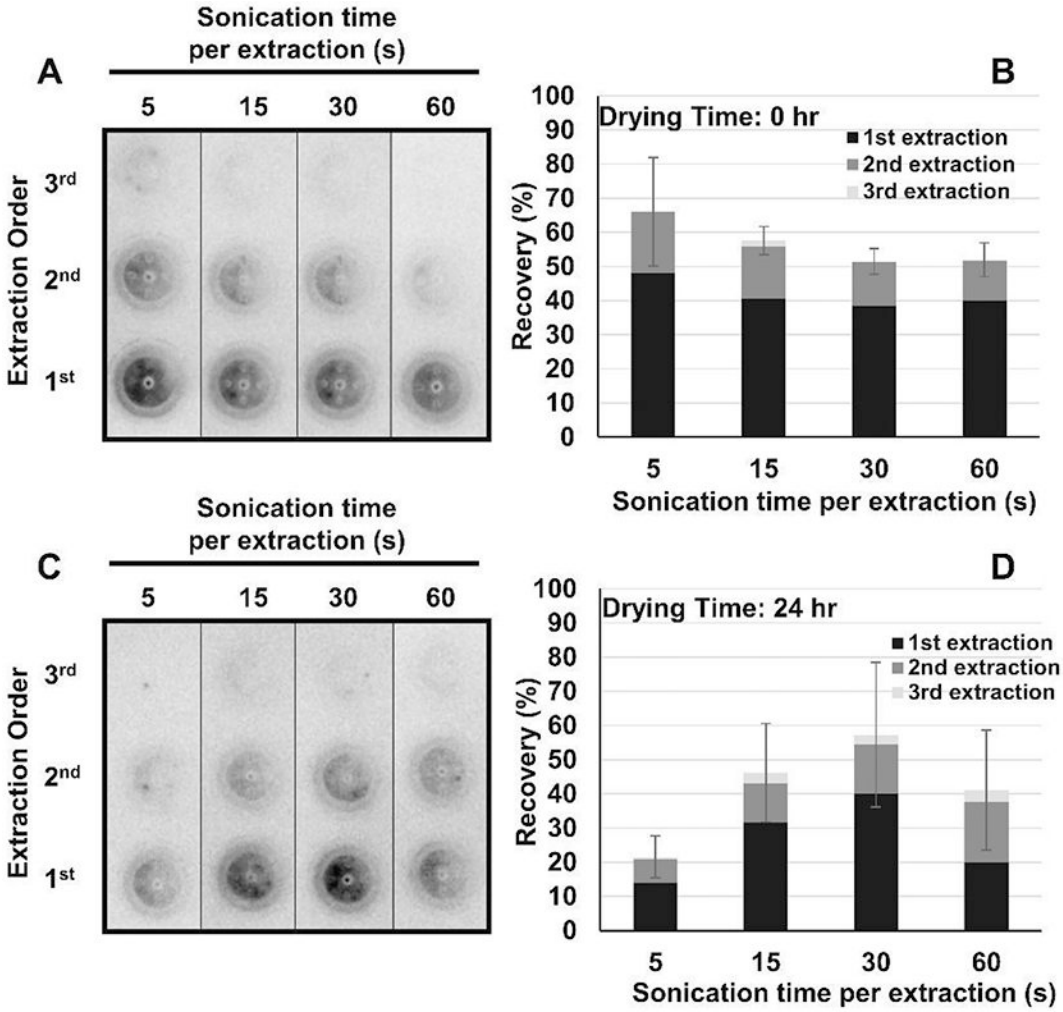

Fig. 2. Recovery of HY TME PrP^{Res} extracted from foam swabs extracted using sonication.
Representative 96-well immunoblots (A, C) of recovered HY TME PrP^{Res} and recovery quantification (B, D corresponding to A, C, respectively) from three sequential extractions (1st, 2nd, 3rd) of foam swabs either undried (0 hr) or dried for 24 hrs at room temperature. Sonication times consist of 1, 3, 6, or 12 cycle(s) of pulse treatment with 5 s of sonication followed by 5 s of incubation. Results of prion recovery are expressed as mean of total recovery ± SEM, n = 4.

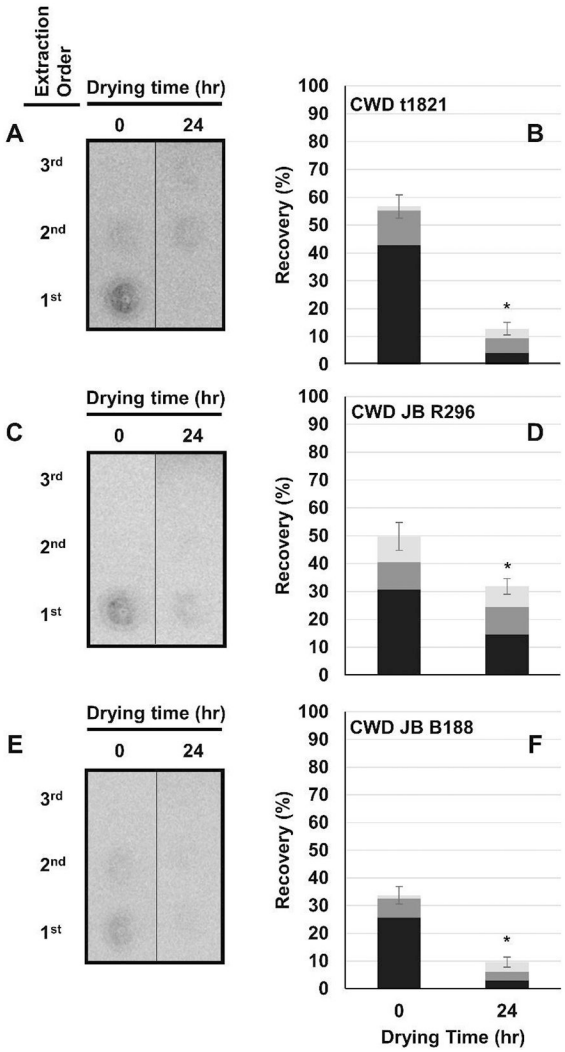

Fig. 3. Recovery of CWD PrP^{Res} extracted from foam swabs with sonication.

Representative 96-well immunoblots (A, C, E) recovered CWD PrP^{Res} and recovery quantification (B, D, F) of from three sequential extractions (1st, 2nd, 3rd) of foam swabs either undried (0 hr) or dried for 24 hrs at room temperature. 15 s (3 cycles of pulse treatment) of sonication was used for each extraction. Results of prion recovery are expressed as mean of total recovery ± SEM, n = 4. * indicates significant difference ($P < 0.05$) between the 24-hr dried sample and respective undried control (0 hr).

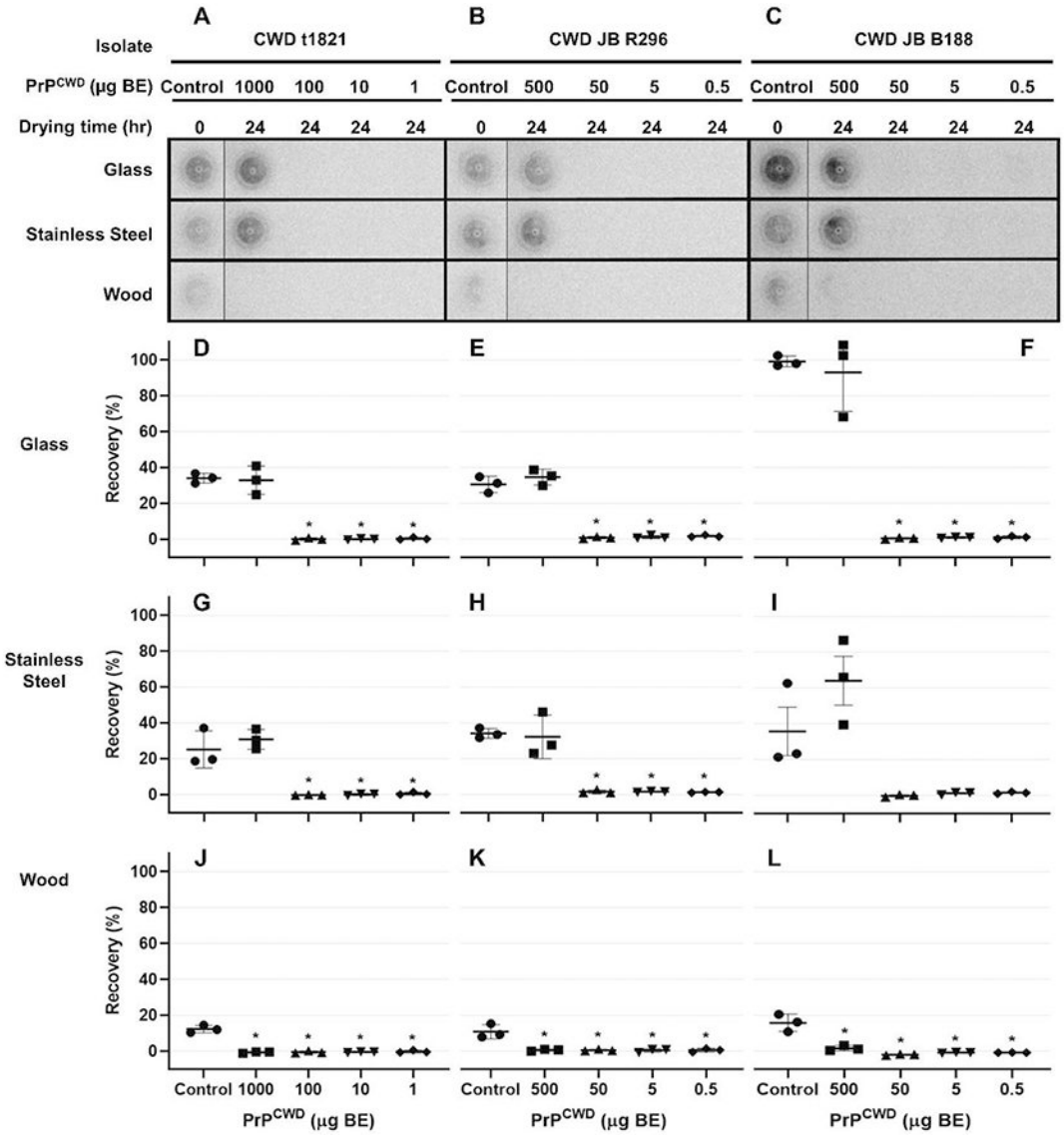

Fig. 4. Recovery of CWD PrP^{Res} from glass, stainless steel, or wood.

Representative 96-well immunoblots (A-C) of recovered CWD PrP^{Res} and recovery quantification (D-L) from foam swabs (combined extract of two sequential extractions for each swab) applied to either undried (control) or 24 hr-dried surfaces at room temperature. 15 s (3 cycles of pulse treatment) of sonication was used for each extraction. For controls, CWD PrP^{Res} at the highest contamination levels were applied. Results of prion recovery are expressed as the mean of total recovery ± SEM, n = 3. * indicates significant difference ($p < 0.05$) between the 24-hr dried sample and respective undried control (0 hr).

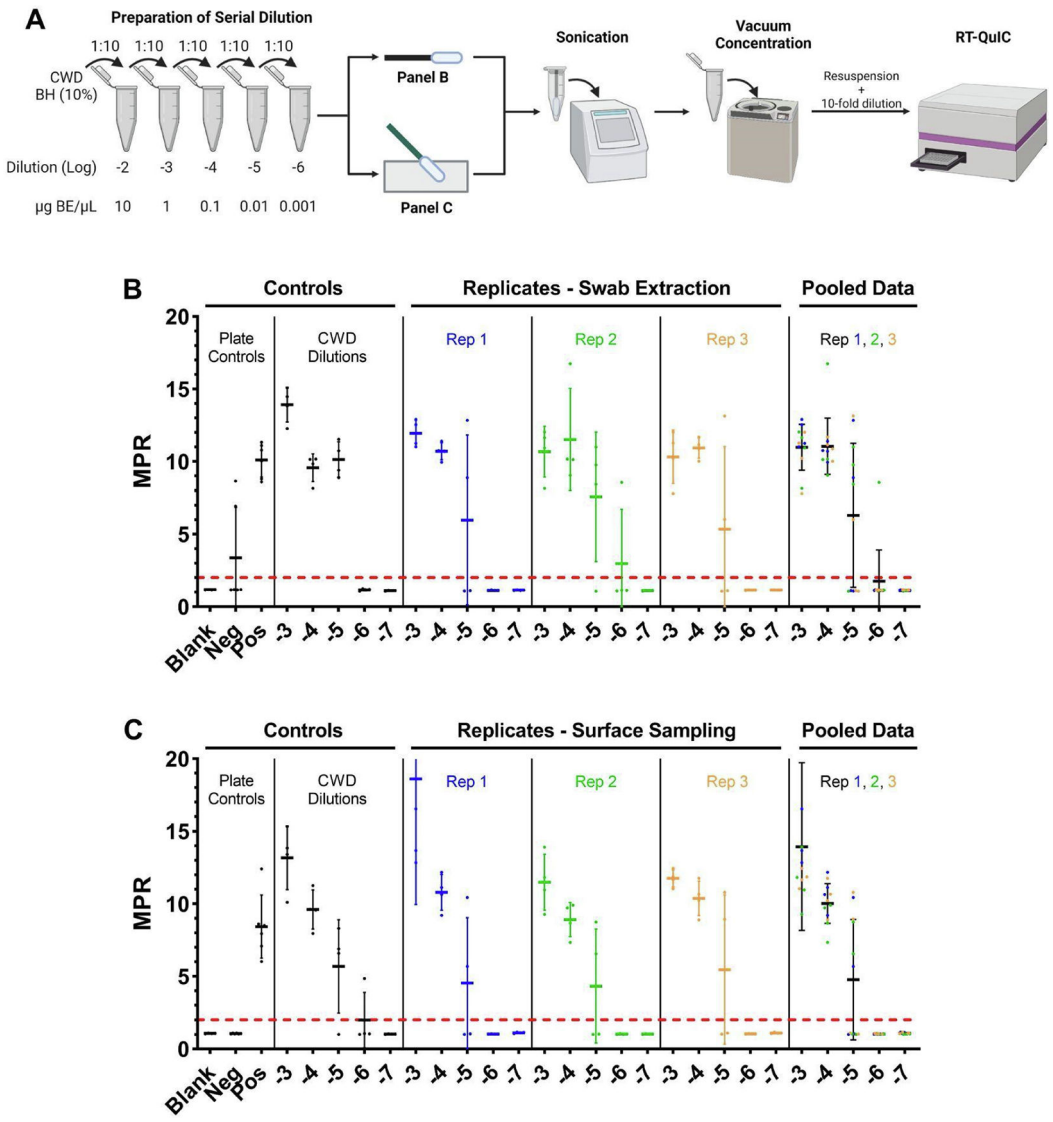

Fig. 5. RT-QuIC detection of CWD prion from swabs and stainless steel – 1st extraction as maxpoint ratio.

(A) RT-QuIC detection methodology for CWD PrPSc; (B) RT-QuIC detection for serial 10-fold dilutions of CWD PrPSc directly applied to swabs that were extracted immediately; (C) RT-QuIC detection for serial 10-fold dilutions of CWD PrP^{Sc} applied to a stainless steel surface that was swabbed after 24-hr drying at 22 °C followed with immediate swab extraction. Swab extract was analyzed for the presence of PrP^{Sc} capable of misfolding recHaPrP to amyloids in the RT-QuIC reaction and expressed as the maxpoint ratio (MPR, mean ± standard deviation) which was determined as the ratio of maximum thioflavin T (ThT) fluorescence in the entire RT-QuIC run to the ThT fluorescence of the starting cycle of RT-QuIC reaction. The threshold of a positive signal was set at 2 (red dashed line).

Table 1

Recovery of PrP^{res} from three CWD isolates from glass, stainless steel, and wood without (0 hr) and with drying (24 hr).

Surface Type	CWD t1821 (%)		CWD JB R296 (%)		CWD JB B188 (%)	
	0 hr[a]	24 hr[b]	0 hr[a]	24 hr[b]	0 hr[a]	24 hr[b]
Glass	34 ± 1[c]	33 ± 5	31 ± 3	35 ± 3	99 ± 2	93 ± 13
Stainless Steel	25 ± 6	31 ± 3	34 ± 2	32 ± 7	36 ± 14	64 ± 14
Wood	12 ± 1	−1 ± 0	11 ± 2	1 ± 0	16 ±3	2 ± 2

[a] Surfaces were contaminated with 1000 μg BE, 500 μg BE, and 500 μg BE for CWD t1821, CWD JB R296, and CWD JB B188 without drying, designated as “control” in Fig. 4.

[b] Surfaces were contaminated with the highest prion loads (equivalent to the “control”) with 24 h of drying.

[c] Total CWD recoveries expressed as mean ± standard error of mean (SEM), n = 3.