December 11, 2019 Webinar:
Chronic Wasting Disease (CWD) Strains

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Regents Professor,
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Center for Infectious Disease Research and Policy, University of Minnesota

cidrap.umn.edu/cwd
Chronic Wasting Disease Resource Center

CWD Response, Research, and Policy Program

The Chronic Wasting Disease (CWD) Response, Research, and Policy Program addresses the transmission of CWD in cervids and its potential for spread to humans and other animal species. The program supports current and reliable information on CWD for the public, including hunters; the medical, veterinary and public health communities; wildlife scientists and managers; and public policymakers.

About CIDRAP's CWD Program  About CWD

Expert Advisory Group

The program includes 54 national and international world-renowned and distinguished leaders in public health, medicine, science, wildlife, and agriculture.

CWD Advisory Group

CWD confirmed in Wyoming deer near elk feeding area

Stephanie Souchery | News Reporter | CIDRAP News | Oct 07, 2019

"Seeing a deer test positive for CWD west of the continental divide again is concerning."

cidrap.umn.edu/cwd
CWD Strains

Debbie McKenzie

Ctr for Prions and Protein Folding Diseases
Biological Sciences,
University of Alberta
Genome Canada: Systems Biology & Molecular Ecology of CWD
Overview

- What is a prion?
- What is CWD?
- Where is CWD found?
- What is a prion strain?
- History of prion strains
- Identification of CWD strains
- Characterization of CWD strains
- How do CWD strains affect transmission, management?
- Environmental contamination by CWD
Glossary

- **PrP**—the prion protein
- **PrP^C**---normal cellular form of the prion protein
- **PrP^Sc**—the abnormal form of the prion protein
  - Aka PrP^CWD, PrP^TSE, PrP^CJD, PrP^BSE
  - Associated with infectivity
- **PrP-res**—the proteinase resistant form of the prion protein
  - May or may not be infectious
- **Prions**—the infectious agent---comprised of PrP^Sc
Prion Diseases

From J Aiken
Characteristics of Prion Diseases

- Spongiform Degeneration
- Transmissible
- Accumulation of PrP\textsubscript{Sc}
- Long Incubation Period
- Extended Preclinical Stage
- Extreme Resistance to Degradation
- Always fatal
- No treatment
Prions (proteinaceous infectious particles) are unprecedented pathogens that seem to be composed of protein only (PrP\textsuperscript{Sc}) and are devoid of nucleic acid. 

(Stanley Prusiner, 1982, Science, 216:136-144)
PrP is encoded by a chromosomal gene.

The *PRNP* gene is copied into mRNA.

mRNA directs the synthesis of PrP\(^C\).

**Molecular biology dogma**

**Prion biology**

PrP\(^C\) is refolded into PrP\(^Sc\).

From H. Wille
Normal protein movements e.g. in an enzyme

Conversion into a dangerous conformation

From H. Wille
Cellular Prion protein (PrPC)  
Mainly α-helix structure  
Proteinase K susceptible
Prion Replication

Initial misfolding of normal form into prion form

Auto-catalytic conversion of normal form into prion form

Prion replication: aggregates fragment to generate new prion “seeds”, each continuing to grow by auto-catalytic conversion of normal protein to the prion form
Chronic Wasting Disease
Alces alces sp.

Cervus canadensis
Scandinavia’s wild cervids

Rangifer tarandus tarandus

Alces alces sp.
Transmission in captive cervids- South Korea

Cervus nipon sp.  
Cervus elaphus

One confirmed case in wild Korean water deer  
Hydropotes inermis

Prion 2016
CWD in North America

Distribution of Chronic Wasting Disease in North America

- CWD in free-ranging populations
- Known distribution prior to 2000 (free-ranging)
- CWD in captive facilities (repopulated)
- CWD in captive facilities (current)

All locations are approximations based on best-available information.
CWD in Alberta
In 2016/2017 (corrected)
- 32,000 deer shot in 4 counties (Richland, Sauk, Iowa and Dane)
- 1 in 8 tested
- 50% prevalence in bucks
Strains

- Sheep
- Cattle
- Mice
- Hamsters
- Humans
- Cervids
A classical scrapie prion isolate + new host species transmission and passaging → Prion strains

"drowsy goat" "scratching goat"

Citation:
A

PrP<sup>C</sup>

PrP<sup>Sc</sup> conformation

1 2 3 4 5 6 7 8 9 10

Propagated by species A

Propagated by species B

Propagated by species C

OVERLAP between permissible PrP<sup>Sc</sup> conformers in species A and B
Transmission between species relatively easy

NO OVERLAP in permissible PrP<sup>Sc</sup> conformers in species A or B and C resulting in a transmission barrier

B

Host A

Host B

Host A

Host B
What makes a “strain” a strain?

- Stable characteristics
  - Incubation period
  - Clinical symptoms
  - Neuropathology
  - Biochemical properties of PrP$^{Sc}$
  - Host range
Transmission of TME into hamsters: selection of two new strains
TME transmission ➔ Hamsters

**Diagram Description:**

- **1st Passage:**
  - Animal #11 (19 kDa) 501 d. (lane 1)
  - Animal #6 (21 kDa) 219 d. (lane 2)

- **2nd Passage:**
  - 167 ± 10 HD (19 kDa) 159 d. (lane 3)
  - 67 ± 3 HY (21 kDa) 66 d.

- **3rd Passage:**
  - 10⁻¹
  - 10⁻⁴
  - 129 ± 4 HD (19/21 kDa) 126 d.
  - 193 ± 11 DY (19 kDa) 181 d.
  - 62 ± 2 HY (21 kDa) 62 d.

- **4th Passage:**
  - 59 ± 2 HY (21 kDa)
  - 151 ± 14 DY (19 kDa)
Hamster-Adapted TME Strains

HYPER (HY)
65 day incubation period
clinical stage: excitable

DROWSY (DY)
165 day incubation period
clinical stage: sleepy
Strains and CWD
CWD1 and CWD2 Strains

- Angers et al. Science 2010
- Elk, mule deer and white-tailed deer agent passaged into “elk” mice
- 2 “strains” identified
  - Long vs short incubation period
  - Different neuropathology
Cervid PrP gene coding polymorphisms

- **White-tailed deer**
  - 95—Gln or His (His associated with longer incubation periods)
  - 96—Gly or Ser (Ser associated with longer incubation periods)
  - 116-Ala or Gly

- **Elk**
  - 132—Met or Leu (Leu provides resistance to CWD)

- **Moose**
  - 209—Met or Ile

- **Mule deer**
  - 225—Ser or Phe (Phe associated with longer incubation periods)

PrPC

Q95H G96S M132L M209I S225F

**Domain location**
- Octarepeat 5
- β-strand 1
- helix 3

Figure adapted from David Westaway
White-tailed deer PrP\textsuperscript{C}

Molecular Dynamics Simulation—Holger Wille & Sara Amidian
Variable amino acid positions

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<tr>
<th>AA95</th>
<th>AA96</th>
<th>AA138</th>
<th>AA226</th>
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<td>K</td>
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<tr>
<td>Q</td>
<td>G</td>
<td>S &gt; N</td>
<td>Q</td>
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Designation

- **wt**
- **Q95H**
- **G96S**
- **Q226K**
- pseudogene

Percent in Wisconsin population

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<td>CWDneg n=153</td>
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<td>0.0</td>
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<tr>
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<td>18.3</td>
<td>24.0</td>
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Variable amino acid positions

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Designation

- wt
- Q95H
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Percent in Wisconsin population

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<tr>
<th></th>
<th>Affected</th>
<th>Non-affected</th>
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<tr>
<td>CWDpos</td>
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<td>n=153</td>
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<td>90.6</td>
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<td>0.3</td>
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<tr>
<td></td>
<td>20.9</td>
<td>18.3</td>
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Incubation periods

Johnson et al. 2011 PLoS ONE
H95/S96 polymorphisms modified the structure of PK-res PrP\textsuperscript{CWD}
Strain Differentiation in tg mice

**96G**

![Graph 96G showing survival days post infection for different CWD allotypes: wt/wt 10% BH, wt/s96 10% BH, wt/h95 10% BH, h95/s96 10% BH.](image)

**96S**

![Graph 96S showing survival days post infection for different CWD allotypes: wt/wt 10% BH, wt/s96 10% BH, wt/h95 10% BH, h95/s96 10% BH.](image)

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**PrP**

![PrP protein structure diagram showing regions labeled as αA, αB, αC, CHO, CHO, and GPI.](image)
Are there additional strains?

- Confirmed:
  - 116G
  - Elk agent

- Potential:
  - Elk agents with different Prnp alleles
  - WTD 020
WTD-06(G116+) & Elk agents cause disease in S96 mice

Open symbols represent tg60 mice with no evident disease signs at the time when the experiment was terminated.

Brain homogenates were treated with 150μg/ml of proteinase K at 37°C during 1 hour.
How do strains arise?

- Cloud hypothesis
- Prnp genotypes
  - Heterologous conversion
- Interspecies transmission
- Other host factors
  - Scrapie in different sheep breeds
A.

PrP<sup>C</sup> → PrP<sup>Sc</sup> conformation

1  2  3  4  5  6  7  8  9  10

<table>
<thead>
<tr>
<th>Propagated by species A</th>
<th>Propagated by species B</th>
<th>Propagated by species C</th>
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<tbody>
<tr>
<td>1  2  3  4</td>
<td>2  3  4</td>
<td>5  6  7</td>
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</table>

**OVERLAP** between permissible PrP<sup>Sc</sup> conformers in species A and B
Transmission between species relatively easy

**NO OVERLAP** in permissible PrP<sup>Sc</sup> conformers in species A or B and C resulting in a transmission barrier

B.

Host A → 4 4 4
Host B → 4 4 4

Host A → 4 4 4
Host B → 2 2 2
Generation of novel CWD strains

- several major foci of CWD in North America
  - Colorado/Wyoming
  - Wisconsin/Illinois
  - Saskatchewan/Alberta
  - Pennsylvania

- Different populations of free-ranging cervids infected
  - Colorado/Wyoming—white-tailed deer, mule deer, elk and moose
  - Wisconsin/Illinois/Pennsylvania—white-tailed deer
  - Saskatchewan/Alberta—primarily mule deer (white-tailed deer, elk and moose)

- Different PRNP polymorphisms
Why are strains important?

- Surveillance
  - Different tissue distribution
  - Different PK-resistance
- Host range
# Differential susceptibility of hamsters and mice to CWD strains

<table>
<thead>
<tr>
<th>CWD</th>
<th>Hamsters</th>
<th>C57BL/6</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
<td>Clinical PrP-res +</td>
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<tr>
<td>Deer wt/wt</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>Deer wt/S96</td>
<td>8</td>
<td>1</td>
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<tr>
<td>Deer H95/wt</td>
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<td>1</td>
</tr>
<tr>
<td>Deer H95/S96</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Alberta Elk Pool(CWD2) (4)</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Uninfected deer</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>
Species Barrier Effect

- Extension of incubation period observed when prion transmitted from one species to another
  - *not necessarily a complete block*

- Incubation period decreases with each passage in the new host species
Species Barrier Effect: Mink and Ferret

- TME
- 27 months
- 18 months
- 12 months
- 4 months
- 27 months
Changing the “Barrier”

CWD
Mule Deer

Ferret

Hamsters
Does CWD transmit to humans?

- NO known cases of human disease linked to CWD
- BUT:
  - As number of infected deer increases, probability increases
  - Different strains may have differential ability to infect
  - Peripheral distribution increases potential risk
- DO NOT EAT untested animals from areas where CWD is high or if your animal tests positive
Chronic Wasting Disease in Cervids: Implications for Prion Transmission to Humans and Other Animal Species

Michael T. Osterholm, Cory J. Anderson, Mark D. Zabel, Joni M. Scheftel, Kristine A. Moore, Brian S. Appleby

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ABSTRACT

Chronic wasting disease (CWD) is a prion-related transmissible spongiform encephalopathy of cervids, including deer, elk, reindeer, sika deer, and moose. CWD has been confirmed in at least 26 U.S. states, three Canadian provinces, South Korea, Finland, Norway, and Sweden, with a notable increase in the past 5 years. The continued geographic spread of this disease increases the frequency of exposure to CWD prions among cervids, humans, and other animal species. Since CWD is now an established wildlife disease in North America, proactive steps, where possible, should be taken to limit transmission of CWD among animals and reduce the potential for human exposure.
Prions are difficult to “kill”

- Chemically Resistant to Inactivation
  - Acids
  - Base
  - Detergent
  - Extreme conditions (1 N NaOH) will inactivate

- Resistant to Standard Sterilization Methods
  - Autoclaving—medical sterilization (120 °C 20 min)
  - Extreme conditions (autoclaving at 134 °C for 1 hour) will inactivate

- UV and gamma irradiation levels that inactivate most bacteria and viruses
Transmission: Direct vs Indirect
Shed CWD Prions can interact with:

- Soils
- Vegetation
Prion-vegetation-soil interactions

Boreal Region

Prions bind to lichen.

Prions transport to lower soil horizons and become unavailable.

Prairie region

Prions wash out of grass.

Prions bind to upper soil horizons.

Bioavailable

From Alsu Kuznetsova
Take home messages

- Transmission of CWD through cervid species expressing different Prnp genotypes results in the selection/generation of new CWD strains

- CWD strains have different biochemical and biological properties (expanded host range)

- CWD can bind to soil and other environmental components---no easy means of decontamination

- All CWD is not the same!
Aiken & McKenzie Laboratory

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Chiye Kim

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Anthony Ness

Danielle Gushue
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  - National Prion Research Program (US)
  - NIH
See you on Tuesday!

Questions?