Beginning the conversation...

Chronic Wasting Disease

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Outline for “the Basics”

- Prions & prion diseases
- History, & characteristics of CWD
- Diagnostic tests
- Transmission & factors influencing
- Surveillance & spread
- Demographic consequences
- Management challenges
Prion:

“Proteinaceous Infectious Particles

- Cellular protein normally produced by all mammals
  - PrP\textsuperscript{C} (Cellular PrP)
  - PrP\textsuperscript{C} is about 250 amino acids
  - PrP\textsuperscript{C} has a coiled, \(\alpha\)-helix shape
  - Soluble, unstable - easily digested by protease K, short half-life
- Does not accumulate in cells
Alternative Fate of Prions

- Converted in the body into disease-causing form
  - PrPres (PrP\text{Sc}, PrP\text{CWD}, PrP\text{BSE})
- Unfolds, then refolds into alternate “β-sheet” form
- Resistant to protease enzymes
- Accumulates into “plaques” or “fibrils”
- Associated with neuronal (especially brain) damage
Normal to Abnormal Conversion

Conversion occurs as a “chain-like” reaction

- Abnormal PrP$_{CWD}$ enters the organism - likely by ingestion
- This “seed” binds to existing PrP$_C$ causes PrP$_C$ to misfold
- Cells detect low PrP$_C$ level, make more
- Cycle continues, PrP$_{CWD}$ accumulates
Prion “Diseases”

Prion diseases, also known as **transmissible spongiform encephalopathies** (TSE’s): group of progressive **neurodegenerative** conditions.

**Animal prion diseases**
- **Scrapie** in sheep and goats, first prion disease to be identified in the 1730s
- **Bovine Spongiform Encephalopathy (BSE)**
- **Chronic wasting disease (CWD)**

**Human prion diseases**
- **Creutzfeldt-Jakob Disease (CJD)**
- **Bovine Spongiform Encephalopathy (BSE)**
- **Kuru**: “to shiver” in Fore language in Papua New Guinea
  - Transmissible mink encephalopathy
  - Feline spongiform encephalopathy
Alternative views to prion theory

Association of Fish and Wildlife Agencies

Statement on Chronic Wasting Disease Etiology

Transmissible spongiform encephalopathies (TSEs) are a family of diseases that have been documented in numerous mammalian species, including cattle, sheep, humans, and members of the deer family (Cervidae or cervids), among others. Decades of scientific research have been dedicated to understanding the cause and treatment of TSEs, including chronic wasting disease (CWD) of cervids. The consensus that has emerged from this research indicates that prions (misfolded proteins) are the causative agents of TSEs, including CWD.
Clinical signs of CWD

Brain spongiform & behaviors

- drastic weight loss ("wasting")
- stumbling, lack of coordination
- listlessness, drooping ears
- excessive thirst or urination, drooling
- lack of fear of people
CWD: What are we up against?
Transmissible spongiform encephalopathy (TSE) of cervids: moose, elk, deer, caribou

Characteristics
- Highly contagious
- No full resistance
- 100% fatal
- Time to death:
  - Deer ~ 1.5-2.5 yrs
  - Elk ~ 4yr

Resists inactivation: ionizing radiation, temperature, formalin, protease and nuclease trt
  → Persists in the environment
CWD Prior to 2000

“Western Disease” with limited scope in N. America

First detection:
- Captive research facility: MD in CO (1967) & WY (1979)
- Free ranging deer, elk & moose in 1980s in CO & WY
CWD in Captive & Free Ranging in 2019

Current status
USA: 25 states
Canada:
Toronto Zoo (1974)
SK: 1996 → 2000
AB: 2002 → 2005
QE: ← 15 km from ON

Korea: 2000 imported elk from Canada
CWD now in Europe

March 2016:
Reindeer in Norway from Nordfjella Mt

June 2016: 2 Moose
> 40,000 surveyed

2018: Finland: Moose

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>CWD+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moose</td>
<td>16,404</td>
<td>4</td>
</tr>
<tr>
<td>Reindeer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Domesticated</td>
<td>21,005</td>
<td>0</td>
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<tr>
<td>Wild</td>
<td>7,433</td>
<td>19</td>
</tr>
<tr>
<td>Red deer - wild</td>
<td>12,659</td>
<td>1</td>
</tr>
<tr>
<td>Roe deer</td>
<td>4,541</td>
<td>0</td>
</tr>
</tbody>
</table>
Detection & Diagnostics

Lymph nodes - earlier detection
Obex (brain) - after neuroinvasion
Detection & diagnostics
Live sampling

Rectal biopsy (RAMALT)

More follicles & earlier detection

Retropharyngeal lymph node biopsy
Detection & diagnostics
Post mortem

- Passive sampling
- Surveillance programs
- Herd reduction programs
Evolution of Diagnostics

- IHC remains the gold standard for CWD confirmation
- Rapid high-throughput ELISA tests developed
- Development of highly sensitive prion replication methods (PMCA & QuIC)

→ Field test of samples

<table>
<thead>
<tr>
<th>Assay-specific conditions</th>
<th>Mechanism of detection</th>
<th>Diagnostic Visualization</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC Acid pretreatment, enzymatic digestion, antigen exposure using fixed, prepared tissues</td>
<td>[Image of IHC mechanism]</td>
<td>[Image of IHC visualization]</td>
</tr>
<tr>
<td>WB Enzymatic digestion of fresh or fixed tissue homogenates; gel separated and adhered to a membrane</td>
<td>[Image of WB mechanism]</td>
<td>[Image of WB visualization]</td>
</tr>
<tr>
<td>EIA Enzymatic digestion or proprietary binding components using fresh tissue homogenates</td>
<td>[Image of EIA mechanism]</td>
<td>[Image of EIA visualization]</td>
</tr>
<tr>
<td>PMCA Sonication, whole brain homogenate, western blot readout</td>
<td>[Image of PMCA mechanism]</td>
<td>[Image of PMCA visualization]</td>
</tr>
<tr>
<td>RT-QuIC Shaking, recombinant protein, real time fluorescent readout</td>
<td>[Image of RT-QuIC mechanism]</td>
<td>[Image of RT-QuIC visualization]</td>
</tr>
</tbody>
</table>

Figure 2. Summary of conventional CWD diagnostic strategies and seeded amplification methods for amplifying CWD prions in vitro. Distinguishing conditions for each assay, as well as illustrative mechanisms of detection and representative diagnostic results are presented. IHC: immunohistochemistry; WB: western blotting; EIA: enzyme immunoassay; PMCA: protein misfolding cyclic amplification; RT-QuIC: real time quaking-induced conversion; * denotes that the structure of amplified products arising from recombinant PrP in RT-QuIC may be different than that produced by PMCA, potentially explaining the loss of infectivity seen with RT-QuIC.

Haley and Richt (2017)
Routes of Transmission
Routes of Transmission

Direct contact: body fluids

Indirect contact: soils/water/veg/surfaces

Bartz et al. 2012
**Dominant Routes of Infection?**

- **Vertical transmission** offspring-adult across placenta occurs but minor role.

- **Direct contacts**: social dynamics 10-20x and habitat influences.

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**Graphs and Figures**

- **Distance between samples (km)**
  - Males
  - Females

- **Pairwise-relatedness categories**
  - Noninfected
  - CWD-infected

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Sources:

- Cullingham et al. 2011
- Schaub er et al. 2015
Dominant Routes of Infection?

- **Environmental**: evidence from penned studies, carcasses/no other deer; bedding
  

- **Potential reservoirs**
  
  - **Soil**: < 2% of diet, feeding, licks
    
    - Prions bind to soils (montmorillonite, clay) can stabilize, may > infectious
    
    - Simulated heating, wetting/drying degraded
    
    - Microbial & lichens can reduce some prion titer
    
    - Humic acids increases, PrP\textsuperscript{CWD} is reduced as HA concentration increases; also may reduce CWD infectivity.

  - **Water**: PrPres BSE in water infectivity in tact
Dominant Routes of Infection?

- Potential reservoirs

Plants

- CWD and other prions can be adsorbed by plants – roots, stems, leaves...so infection pathway
- Lichen extracts may degrade or inactivate prions
- Norway put restrictions on hay and straw imports from CWD areas in 2018

CWD contamination on plants via urine, feces, or saliva, or root uptake of plants growing in CWD-infected soils present risk of spreading the disease. (Adapted from Pritzkow, et al, Cell Reports, 2015.)
Dominant Routes of Infection?

- Potential animal vectors

Experimentally inoculated animals had infectious prions in feces far ranging …but modified?

Nichols et al. 2015, Miller 2008
Dominant Routes of Infection?

- Studies/experiments show the **proof of principle** in exposure
- Realistic modeling of direct & indirect is complex
- Importance of environment **over time**

![Relative CWD Infection Graph]

- X-axis: Time
- Y-axis: Relative CWD Infection
- Lines: Total, Direct, Environment
Prevalence & Infection in Space

Wisconsin

- Two outbreaks in WI and IL are merging
- Diffuses outward with highways and rivers slowing spread

Alberta

- Edge from SK moving along rivers
86% CWD positive cases since 2005 (n = 919) in AB have been mule deer.
Prevalence & Infection

Genotypes: all are susceptible to CWD

- Some slower disease progression
- Potential evolution of resistance likely will take decades; outcome still unknown
- Spatial structure?
- Will resistant genotypes have reduced fitness?
Prevalence & Infection: Sex

- Adult males 3-4x > infected adult females
- CWD+ males > mortality than CWD- males
- CWD+ males > mortality than CWD+ females

Samuel and Storm 2016
### Weighted Surveillance

**TABLE 2** Chronic wasting disease (CWD) log infection ratios ($\beta$), infection ratios ($w$), surveillance weights ($R$), approximate number of samples ($n$) needed to achieve $\Pr(\pi_{ref} \leq 0.01) \geq 0.95$, and precision ($SD$) from using white-tailed deer harvest data from 2003 to 2010 in the CWD management zone of Wisconsin. Hunter-harvested yearling males are the reference class.

<table>
<thead>
<tr>
<th>Mortality source</th>
<th>$\beta$ (SD)</th>
<th>$w$ (SD)</th>
<th>$R$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical suspect; community reported</td>
<td>3.549 (0.208)</td>
<td>35.530 (7.426)</td>
<td>33.333</td>
<td>9</td>
</tr>
<tr>
<td>Clinical suspect; hunter reported</td>
<td>2.270 (0.209)</td>
<td>9.888 (2.062)</td>
<td>9.091</td>
<td>33</td>
</tr>
<tr>
<td>Found dead</td>
<td>2.204 (0.337)</td>
<td>9.575 (3.198)</td>
<td>7.317</td>
<td>41</td>
</tr>
<tr>
<td>Sharpshot adult male</td>
<td>1.294 (0.246)</td>
<td>3.757 (0.9193)</td>
<td>3.297</td>
<td>91</td>
</tr>
<tr>
<td>Hunter-harvested adult male</td>
<td>1.169 (0.105)</td>
<td>3.237 (0.3433)</td>
<td>3.226</td>
<td>93</td>
</tr>
<tr>
<td>Sharpshot adult female</td>
<td>0.527 (0.224)</td>
<td>1.736 (0.3895)</td>
<td>1.563</td>
<td>192</td>
</tr>
<tr>
<td>Hunter-harvested adult female</td>
<td>0.277 (0.113)</td>
<td>1.328 (0.1508)</td>
<td>1.304</td>
<td>230</td>
</tr>
<tr>
<td>Hunter-harvested yearling male</td>
<td>–</td>
<td>–</td>
<td>1.000</td>
<td>298</td>
</tr>
<tr>
<td>Sharpshot fawn male</td>
<td>–0.144 (0.410)</td>
<td>0.939 (0.378)</td>
<td>0.625</td>
<td>480</td>
</tr>
<tr>
<td>Vehicle collision</td>
<td>–0.381 (0.806)</td>
<td>0.898 (0.641)</td>
<td>0.216</td>
<td>1391</td>
</tr>
<tr>
<td>Hunter-harvested yearling female</td>
<td>–0.142 (0.147)</td>
<td>0.877 (0.130)</td>
<td>0.850</td>
<td>353</td>
</tr>
<tr>
<td>Sharpshot yearling male</td>
<td>–0.279 (0.548)</td>
<td>0.868 (0.449)</td>
<td>0.432</td>
<td>695</td>
</tr>
<tr>
<td>Sharpshot fawn female</td>
<td>–0.534 (0.539)</td>
<td>0.669 (0.337)</td>
<td>0.347</td>
<td>865</td>
</tr>
<tr>
<td>Sharpshot yearling female</td>
<td>–0.957 (0.808)</td>
<td>0.506 (0.367)</td>
<td>0.121</td>
<td>2475</td>
</tr>
<tr>
<td>Hunter-harvested fawn female</td>
<td>–2.026 (0.485)</td>
<td>0.147 (0.068)</td>
<td>0.084</td>
<td>3570</td>
</tr>
<tr>
<td>Hunter-harvested fawn male</td>
<td>–4.016 (1.276)</td>
<td>0.032 (0.032)</td>
<td>0.001</td>
<td>250600</td>
</tr>
<tr>
<td>$\tau$ - year effect</td>
<td>0.202 (0.017)</td>
<td>1.224 (0.0202)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Infection Rate | Surveillance weight | $n$ | needed

Jennelle et al. 2018
Consequences For Bucks?

- Average longevity for a buck without CWD is 1 more year
- Average longevity for a buck with CWD is 4 more months
- Fewer and younger bucks
Consequences For Does?

- Average longevity for a doe without CWD is 2 more years.
- Average longevity for a doe with CWD is 6 more months.
- Decreasing deer populations.

Samuel and Storm 2016
Population Impacts?

- **Mule deer (Miller et al. 2008) - Colorado**
  - Pop growth < 1 unhunted; prev ~25% CWD; higher mortality in CWD infected deer

- **RMNP Elk (Monello et al. 2014) - Colorado**
  - Pop growth = 1.0; prev > 13% Pop growth < 1; higher mortality in CWD infected elk

- **White-tailed deer (Edmunds et al. 2016) - Wyoming**
  - Pop growth = 0.9; Females (42%) < Males (29%); CWD infected deer 4.5 times higher mortality

- **Mule deer (DeVivo PhD 2015) - Wyoming**
  - Pop growth = 0.74; Males (50%) > Females (30%); CWD infected deer 2.8 times higher mortality
The “super wicked” problem

- Slow disease progression
- Complexities of transmission that may change with state
- Environmental persistence
- Genotype resistances
- CWD strains
- No vaccine
- Management efforts require long-term political & financial commitments

“The emergence of chronic wasting disease affecting mule deer, white-tailed deer, and elk is arguably the most important issue in the management of free-living cervids in North America.”

Expert Scientific Panel, 2004
Questions?
Beginning the conversation...

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