Systematic evaluation of the zoonotic potential of different CWD isolates.

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Disclosure

Dr. Rodrigo Morales is an inventor in a patent application related to the Protein Misfolding Cyclic Amplification technology
The CWD problem

✓ CWD affect deer, elk, moose and other cervids both captive and wild range.

✓ CWD affects almost all cervidae in experimental conditions

✓ CWD was first described in 1967 with infected mule deer on a breeding farm in Colorado. Now has been detected in 22 states of the USA, Canada, South Korea, Norway and Finland.

✓ Clinical signs include wasting, change of behavior, head tremors, excessive drinking and urination, reduced eating, walking repetitive path, pneumonia, disorientation, lose control of bodily function and death.

✓ Disease mostly affects adults 17 months to 15+ years. Clinical duration >1 year. Time between exposure to infectious material and clinical disease is at least 17 months.

✓ There is no treatment or vaccine available. Measures to limit further spreading include quarantine, depopulation and decontamination of farms. It is unclear how efficient these measures are.

✓ Cervid farming industry was estimated to represent $18.1 billion US dollars in 2011 (only related to hunting).
Some *cervidae* affected by CWD

- White-tailed deer
- Mule deer
- Moose
- Elk
- Red deer
- Sika deer
- Caribou
- Muntjac deer
Biological samples relevant to CWD transmission and diagnosis

- Brain
- Saliva
- Blood
- Antlers
- Retropharyngeal lymph nodes
- Placenta
- Meat
- Urine
- Rectal mucosa lymph nodes
- Feces
Role of the environment in prion transmission

Healthy animals → Prions enter the environment → Prions are retained and buildup in the environment → Prions are spread → Animals exposed to contaminated materials → Prion infected animals

Prions enter the environment
CWD detection in blood of pre-symptomatic white-tailed deer

Detection of Prions in Blood of Cervids at the Asymptomatic Stage of Chronic Wasting Disease

Carlos Kramm, Sandra Pitzhorn, Adam Lyon, Tracy Nichols, Rodrigo Morales & Claudio Soto

In addition, we successfully implemented PMCA for the pre-symptomatic detection of CWD in saliva, feces, semen and sexual related tissues, as well as environmental components.

Summary of results obtained in a blind study of detection of infectious prions in blood samples from white-tailed deer (WTD) at various stages of CWD.

<table>
<thead>
<tr>
<th>CWD Status</th>
<th>Total no. of deer</th>
<th>PMCA positive</th>
<th>Correct diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic WTD (B+ LN+)</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Asymptomatic WTD (B+ LN+)</td>
<td>48</td>
<td>46</td>
<td>96%</td>
</tr>
<tr>
<td>Asymptomatic WTD (B- LN+)</td>
<td>34</td>
<td>18</td>
<td>53%</td>
</tr>
<tr>
<td>Negative WTD (B- LN-)</td>
<td>13</td>
<td>0</td>
<td>100%</td>
</tr>
</tbody>
</table>

1Samples were declared as positive in PMCA if at least one of the replicates gave a protease-resistant PrPSc signal in any of the PMCA rounds analyzed.

Kramm et al. 2017. Scientific Reports
Mimicking inter-species transmissions of prions by PMCA

Crossing the Species Barrier by PrP^{Sc} Replication In Vitro Generates Unique Infectious Prions

Joaquin Castilla, 1 Dennisse Gonzalez-Romero, 1, 4 Paula Saa, 1, 5, 6 Rodrigo Morales, 1, 2, 4 Jorge De Castro, 1 and Claudio Soto 1, 4

Cell

Barria et al. 2011. JBC
**In vitro** evaluation of the zoonotic potential of different animal isolates

PMCA reproduced expected inter-species transmissions of animal prion diseases in “humanized” models
**Prnp** polymorphisms in white-tailed deer

![Diagram of Prnp polymorphisms in white-tailed deer](image)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Percentage in Deer Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( CWDP^\text{mm} )</td>
</tr>
<tr>
<td>wt</td>
<td>90.6</td>
</tr>
<tr>
<td>G96S</td>
<td>8.9</td>
</tr>
</tbody>
</table>

Modified from Johnson et al., 2006

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Modified from Kelly et al., 2008
PrP 96GG – CWD prion isolates have greater potential to template misfolding of human PrP\(\text{C}\) compared to its PrP 96SS counterpart
PrP 96SS CWD adapted in PrP 96GG substrate has a low potential to misfold human PrPC. Serially adapted PrP 96SS prions in homologous substrate has a greater zoonotic potential.
Assessing inter-species CWD transmissions by PMCA

Adapted CWD PrP 96SS isolates have higher inter-species transmission potentials compared to their PrP 96GG counterparts.
Conclusions and Future Directions

- We have developed a highly sensitive and specific CWD-PMCA platform to be used as a diagnostic tool.
- Current PMCA set up allow us to mimic relevant prion inter-species transmission events.
- Polymorphic changes at position 96 of the prion protein apparently alter strain properties and, consequently, the zoonotic potential of CWD isolates.
- Inter-species and inter-polymorphic $\text{PrP}^C \rightarrow \text{PrP}^\text{Sc}$ conversions further increase the spectrum of CWD isolates possibly present in nature.
- CWD prions generated in 96SS $\text{PrP}^C$ substrate apparently have greater inter-species transmission potentials.
- Future experiments will explore the zoonotic potential of CWD prions along different adaptation scenarios, including inter-species and inter-polymorphic.
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The **CJD Foundation Grant**, contributed by The Families of the CJD Foundation