Webinar Introduction

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Prion and Public Health Office

July 10, 2017

National Center for Emerging and Zoonotic Infectious Diseases

Division of High-Consequence Pathogens and Pathology

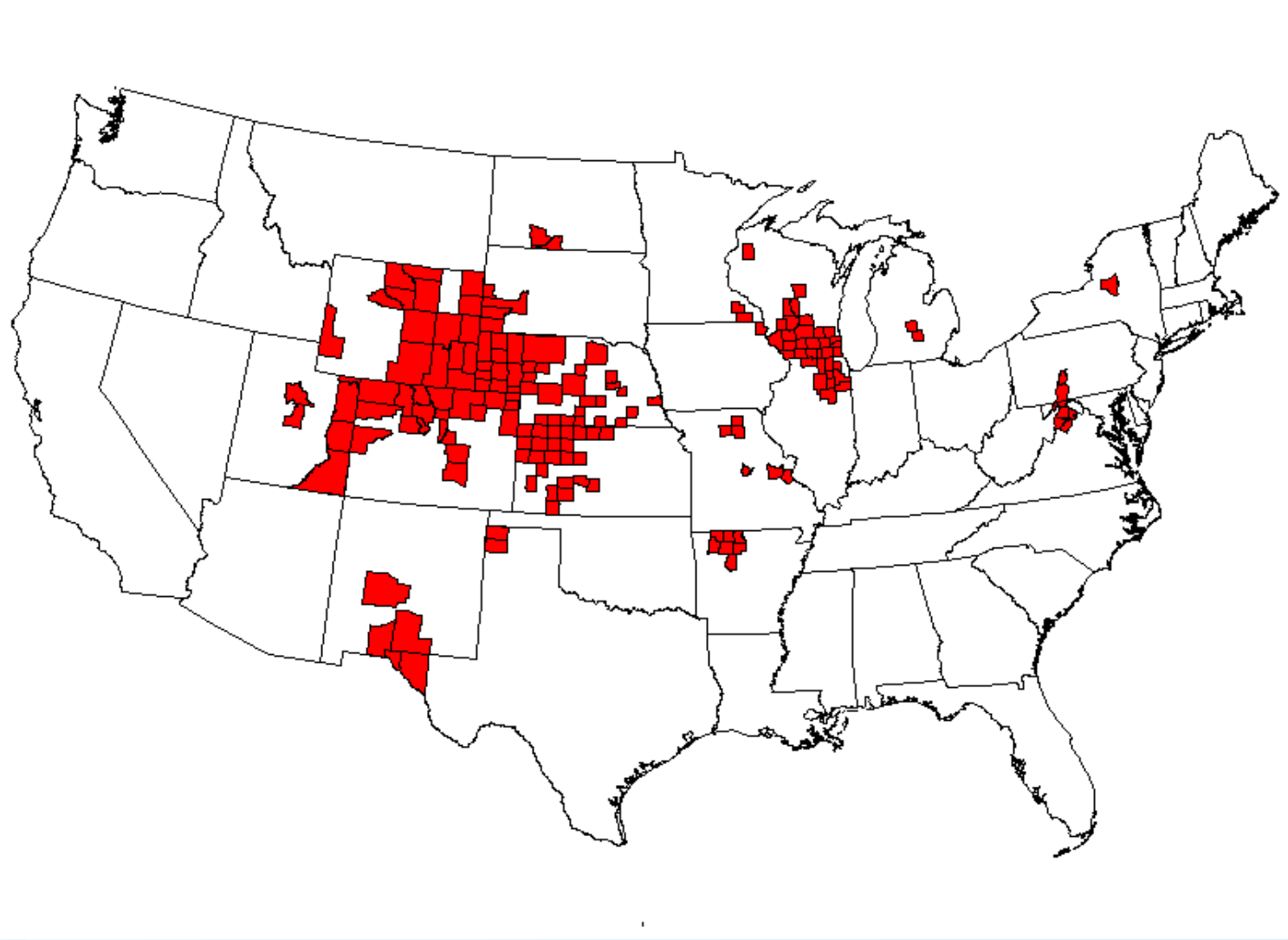
Today's Presentation

- Prion 2017 conference presentation by Dr. Stefanie Czub
  - Suggested great caution regarding human exposure to CWD
  - Convinced CDC attendees that there was a need for the CWD community to be aware of findings
- Thanks to:
  - NASPHV
  - Meri Phillips
  - Call participants
  - Dr. Czub

Human Exposure to CWD

- CDC recommends that humans avoid exposure, if possible, to transmissible spongiform encephalopathies (TSEs).
- Foodborne Diseases Active Surveillance Network (FoodNet) 2006-2007 population survey (n=17,372)
  - 18.5% of respondents had hunted deer or elk
  - 67.4% had eaten deer or elk meat
- CWD outbreak among free-ranging cervids expanding
  - CWD in free-ranging cervids in 21 states and 2 Canadian provinces
  - Increasing number of people at risk of being exposed to CWD

Chronic Wasting Disease Among Free-Ranging Cervids by County, United States, June 2017



Surveillance

- CDC conducts surveillance to help monitor for human CWD.
  - Investigates unusual cases of human prion disease
  - Attempts to identify prion disease cases among persons with an increased risk of exposure to the CWD agent
- To date, we have no strong epidemiological evidence for the occurrence of any case of human CWD.
  - Potentially long incubation periods
  - United Kingdom experience with BSE

The Study

- **Dr. Czub's study:**
  - Uses cynomolgus macaques - close genetic similarity to humans
  - Mimics the types of exposure humans have with CWD
  - Increases concerns about possible CWD risk to humans
  - Is unlikely to be repeated by others in the near future due to prohibitive cost

So without further delay…

Dr. Czub

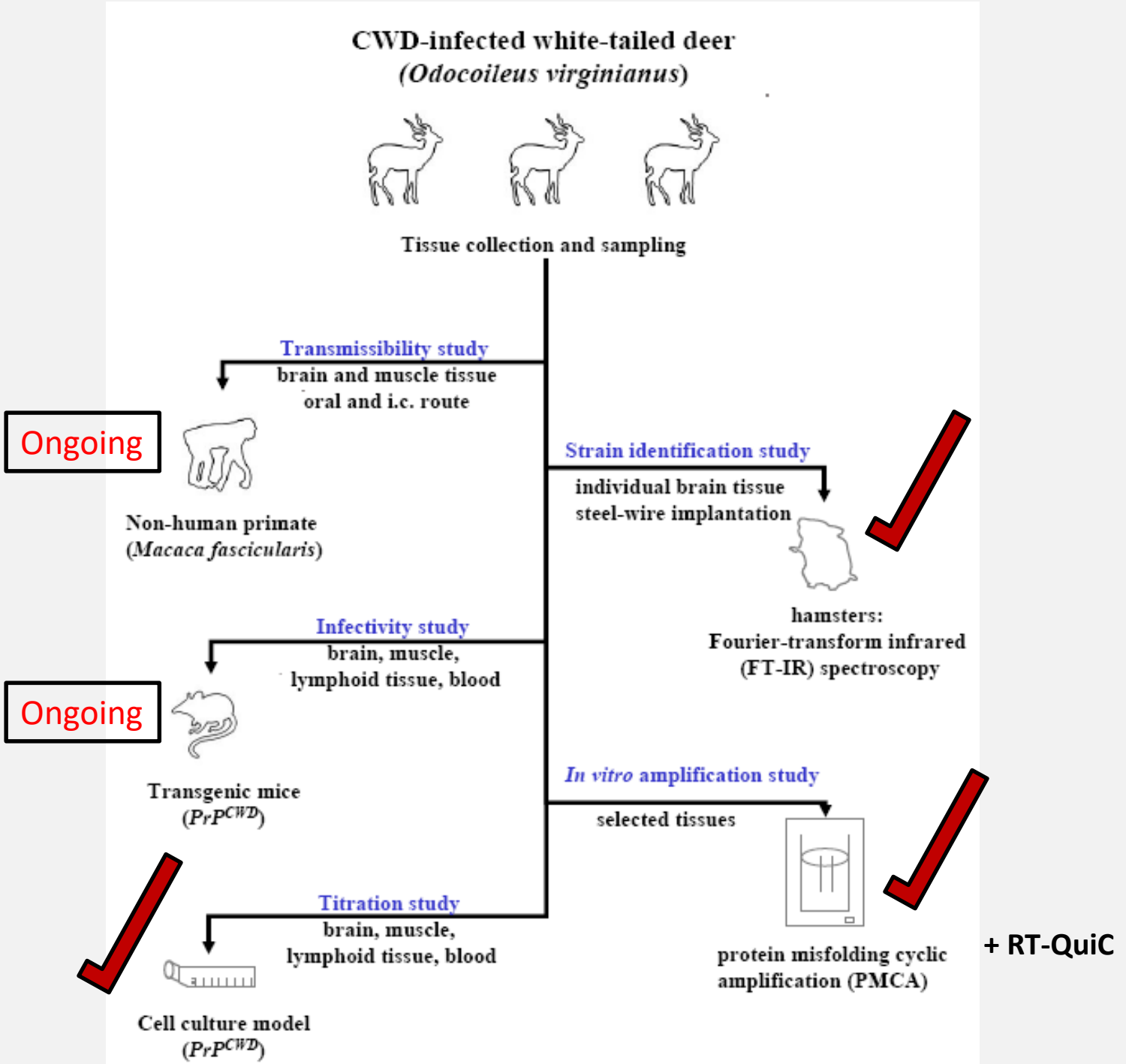
Frist evidence of intracranial and peroral transmission of Chronic Wasting Disease (CWD) into Cynomolgus macaques: *a work in progress*

Stefanie Czub, Walter Schulz-Schaeffer, Christiane Stahl-Hennig, Michael Beekes, Hermann Schaetzl and Dirk Motzkus

CSTE/NASPHV WEBINAR, JULY 10 2017

Project Specifics

- Start: April 2009
- Funding Agency: Alberta Prion Research Institute (APRI), Alberta Livestock & Meat Agency (ALMA)
- Funding volume: > $ 7.9 million
- Project lead: Dr. Stefanie Czub UCVM/CFIA
- Collaborators: Drs. Dirk Motzkus/C. Stahl-Hennig (German Primate Center); Walter Schulz-Schaeffer (German CJD Reference Lab/UMG); Michael Beekes (Robert-Koch-Institute); Hermann Schaetzl (UCVM/UofC)


CWD-infected white-tailed deer
(Odocoileus virginianus)
Tissue collection and sampling
Transmissibility study
brain and muscle tissue
oral and i.c. route
Ongoing
Non-human primate
(Macaca fascicularis)
Strain identification study
individual brain tissue
steel-wire implantation
hamsters:
Fourier-transform infrared
(FT-IR) spectroscopy
Infectivity study
brain, muscle,
lymphoid tissue, blood
Ongoing
Transgenic mice
(PrPCWD)
In vitro amplification study
selected tissues
protein misfolding cyclic
amplification (PMCA)
+ RT-QuiC
Titration study
brain, muscle,
lymphoid tissue, blood
Cell culture model
(PrPCWD)

CWD Transmission into non-human Primates

- Project Goal
  - Transmissibility - to investigate the zoonotic potential of CWD (1/5 goals)
  - Clinical endpoint

- Experimental Design
  - 21 macaques, different routes of challenge (n=18). 3 mock controls
  - Intracranial – proof of concept
  - Per oral – risk via consumption
  - Skin scarification – risk via field dressing
  - Intravenous – risk via blood transfusion

Animals & challenge material

- 21 female cynomolgus macaques of Mauritian origin (age-matched) (Noveprim/Spain)
- With wild-type PrP , homozygous for methionine at codon 129 (PCR)

- *CWD WTD challenge material*:

  confirmed disease status (rapid tests, PET-Blot)

  2 distinct CWD isolates by biochemical (limited proteolysis – different Pk, pH & Guanidinium hydrochloride concentrations); conversion activity (PMCA); & structural analyses (Fourier transform infrared spectroscopy & atomic force microscopy) (M. Daus & M. Beekes, Prion 2010, p 133)

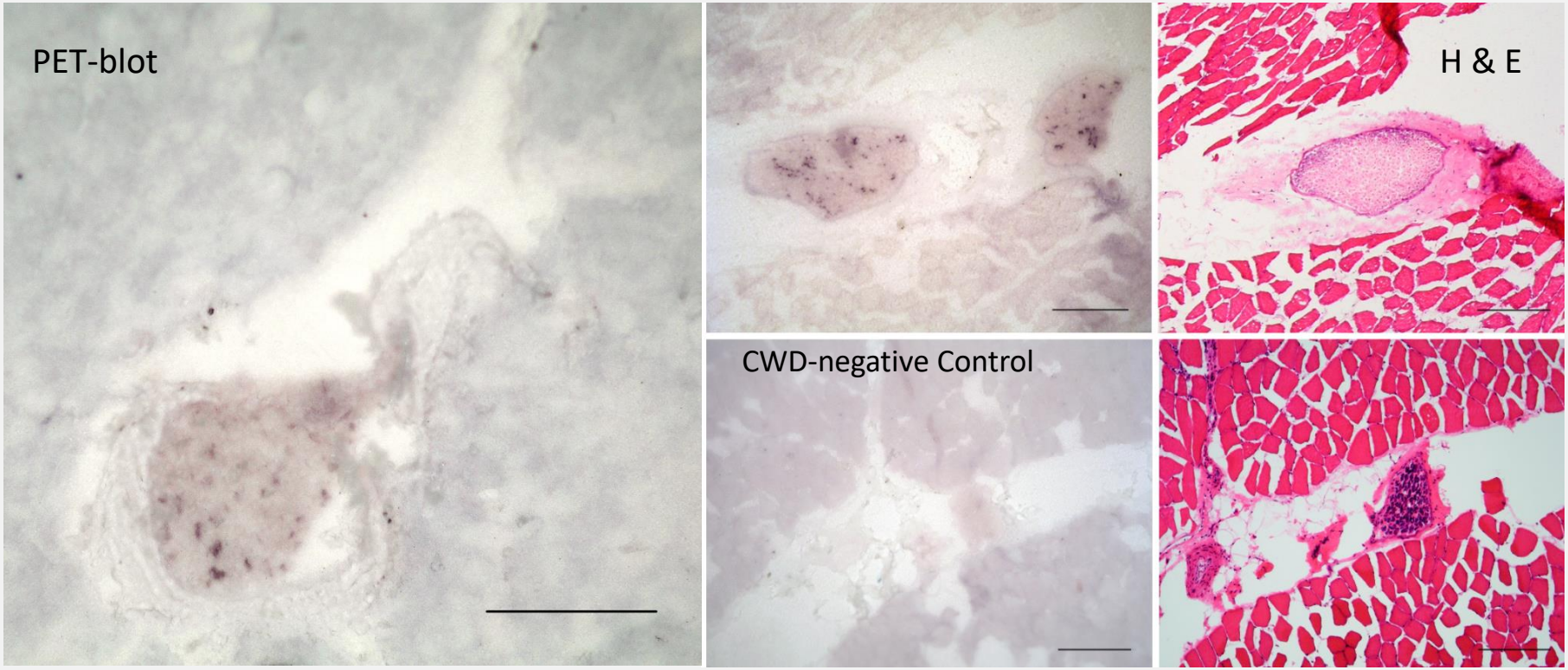
- *CWD Elk challenge material*:

  confirmed disease status (rapid tests, IHC). Brain pool from 3 elk (132 MM homozygous) with clinical disease; pool determined to be CWD2

  Titer $10^{7.2}$ i.c. ID50/g brain Tg(CerPrP-M132)1536 & titer $10^{7.0}$ i.c. ID 50/g brain (CerPrP-E226)5037 (Bian et al, JVirol, 2010)

- *Macaque-macaque blood transfusion:* non-clinical macaques as blood donors, challenged with CWD MD, elk & WTD. Infectious titers of challenge material ranged 1.0 X $10^{6.0}$ i.c. ID 50/g to 2.0 x $10^{9.0}$ i.c. ID 50/g brain in deer tg mice (Race et al, Emerging Infect Dis, 2009)

PrP^{CWD} presence in WTD skeletal muscles (Pet-Blot)



mAB: P4, bar = 250 µm

Presence and Seeding Activity of Pathological Prion Protein (PrP^{TSE}) in Skeletal Muscles of White-Tailed Deer Infected with Chronic Wasting Disease

Daus ML, Breyer J, Wagenfuehr K, Wemheuer WM, Thomzig A, Schulz-Schaeffer WJ, Beekes M., PLoS One 2011

Animal ID	Date of inoculation	Route of inoculation	Inoculum	Date of autopsy	Lymphnode biopsies	Years pi (05/2017)
AU242	2009/10/27	Ic steel wire	mock control material	2015/06/30	-	**5.7**
AU153	2009/11/24	Ic steel wire	CWD WTD	2012/09/10	-	**2.8**
AU500	2009/10/27	Ic steel wire	CWD WTD		-	**7.6**
AU519	2009/11/24	Ic steel wire	CWD WTD	**2015/01/21**	-	**5.2**
AU308	2010/11/09	Ic steel wire	CWD elk		-	**6.6**
AU389	2010/11/09	Ic steel wire	CWD elk	**2015/05/04**	-	**4.5**
AU520	2010/11/09	Ic steel wire	CWD elk	**2017/02/22**	-	**6.3**
AU406	2009/07/07	Ic	mock control		-	**7.6**
AU408	2009/07/07	Ic	10 mg CWD WTD	**2016/01/28**	-	**6.5**
AU469	2009/07/07	Ic	10 mg CWD WTD	**2016/06/06**	-	**6.9**
AU398	2009/05/27	Skin scarification	1 ml 10 % CWD WTD		-	**7.10**
AU451	2009/05/20	Skin scarification	1 ml 10 % CWD WTD		-	**7.10**
AU315	2009/04/29 - 2010/07/20	oral	5 x 2mg mock control (WTD brain)		-	**8**
AU467	2009/04/29 - 2010/07/20	oral	5 x 2 g CWD WTD brain	**2015/03/04**	-	**5.8**
AU243	2009/04/29 - 2010/07/20	oral	5 x 2 g CWD WTD brain		-	**8**
AU316	2009/09/14 - 2012/11/02	oral	~5 kg CWD-WTD (repeatedly)	**2017/03/10**	-	**7.4**
AU385	2009/09/14 - 2012/11/06	oral	~5 kg CWD-WTD muscle (repeatedly)	**2015/12/09**	-	**6.2**
AU501	2009 /09/14- 2012/11/06	oral	~5 kg CWD-WTD muscle (repeatedly)	**2015/02/01**	-	**5.4**
AU456	2009/11/12	blood transfusion	14 ml plasma/buffy coat {RML#128+616 (elk)}			**7.5**
AU382	2009/11/16	blood transfusion	14 ml plasma/buffy coat {RML#144+116 (WTD}			**7.5**
AU390	2009/11/16	blood transfusion	9.5 ml plasma/buffy coat {RML#135 (mule deer)}			**7.5**

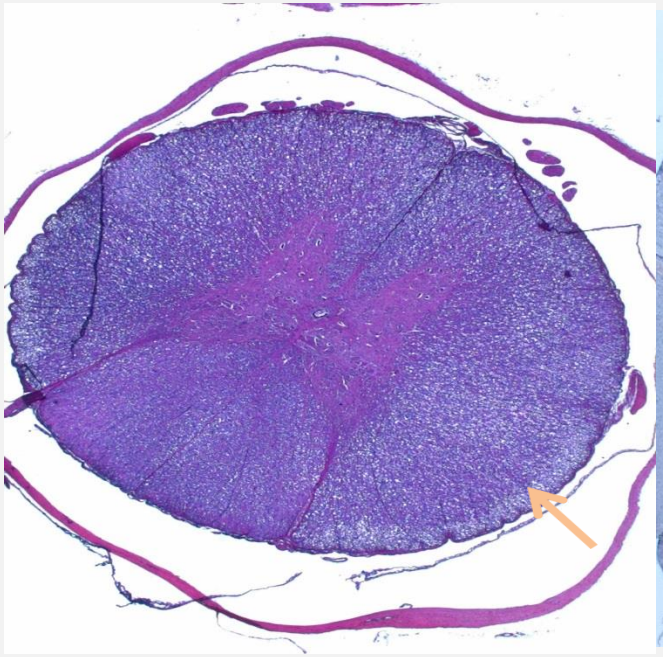
Animal info

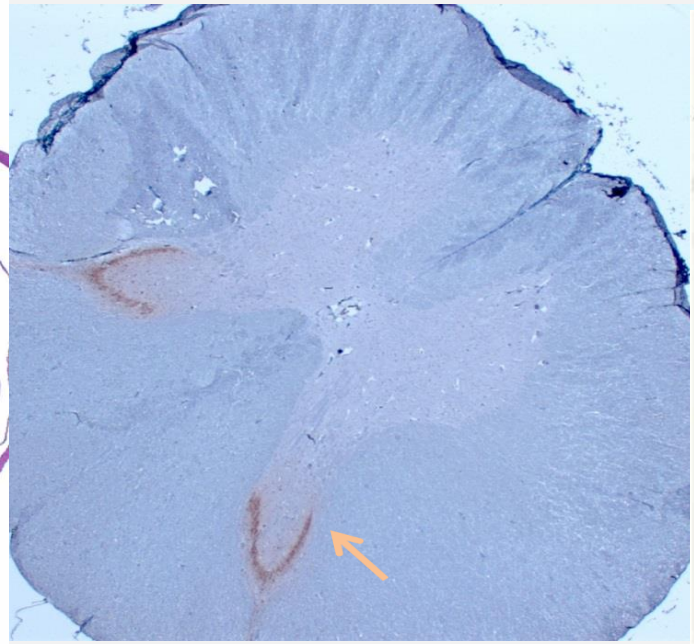
Animal #	AU242	AU153	AU389	AU519	AU520	AU408	AU469	AU467	AU501	AU385	AU316
Years p.c.	**5.7**	**2.8**	**4.5**	**5.2**	**6.3**	**6.5**	**6.9**	**5.8**	**5.4**	**6.2**	**7.4**
Route	**i.c.** steel wire	**i.c.** steel wire	**i.c.** steel wire	**i.c.** steel wire	**i.c.** steel wire	**i.c.**	**i.c.**	**oral**	**oral**	**oral**	**oral**
Inocul.	**Mock (-)**	**WTD+**	**Elk+**	**WTD+**	**ELK+**	**WTD+ pool**	**WTD+ pool**	**WTD+ brain**	**WTD+ muscle**	**WTD+ muscle**	**WTD+ muscle**
Clinical present.	**wasting**	**no (pm)**	**anxiety ataxia tremor wasting**	**no (pm)**	**wasting**	**no**	**wasting**	**wasting** (died p. anest.)	**anxiety ataxia tremor wasting**	**apathy ataxia tremor wasting**	Ileus
Clinpath	**Glucose** ↑	**no**	**Glucose** ↑	**no**	**Glucose & HbA1c** ↑	**Glucose** ↑	**Glucose & HbA1c** ↑	**no**	**no**	**Glucose** ↑	**no**

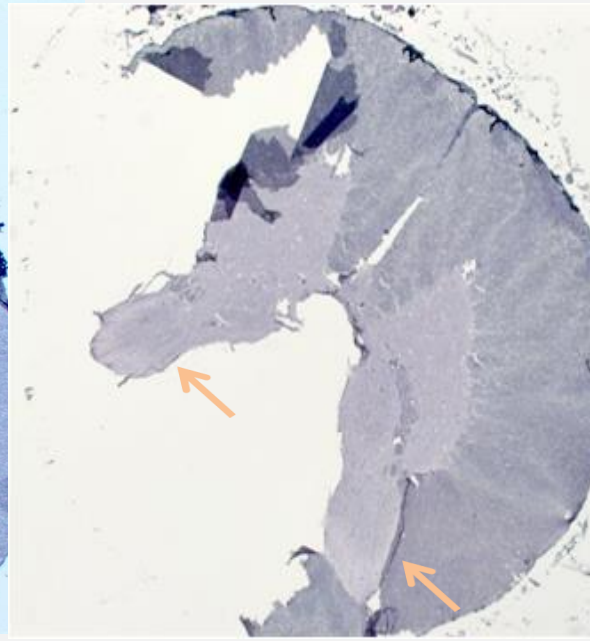
Morphological assays

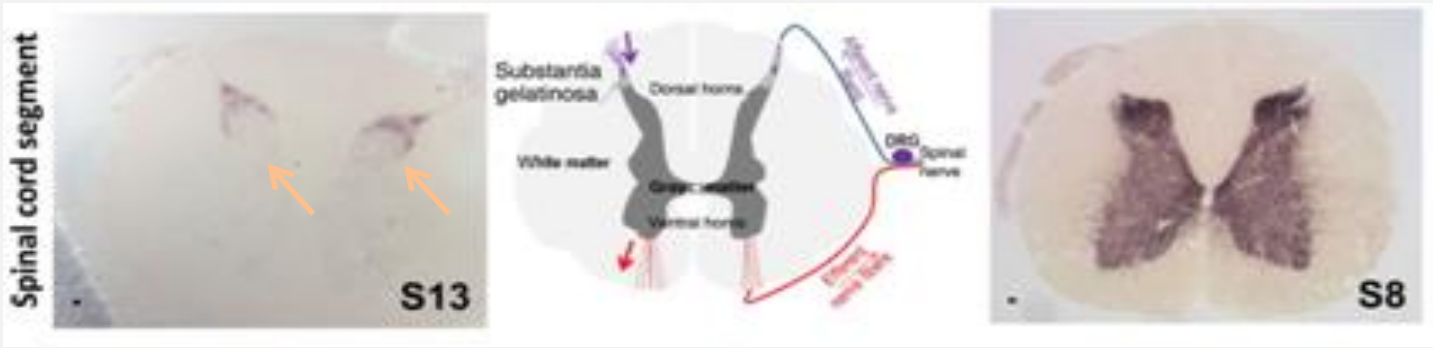
IMMUNOHISTOCHEMISTRY & HISTOPATHOLOGY

Proof-of-concept: i.c. sw*; CWD elk, 4.5 years (AU389)*







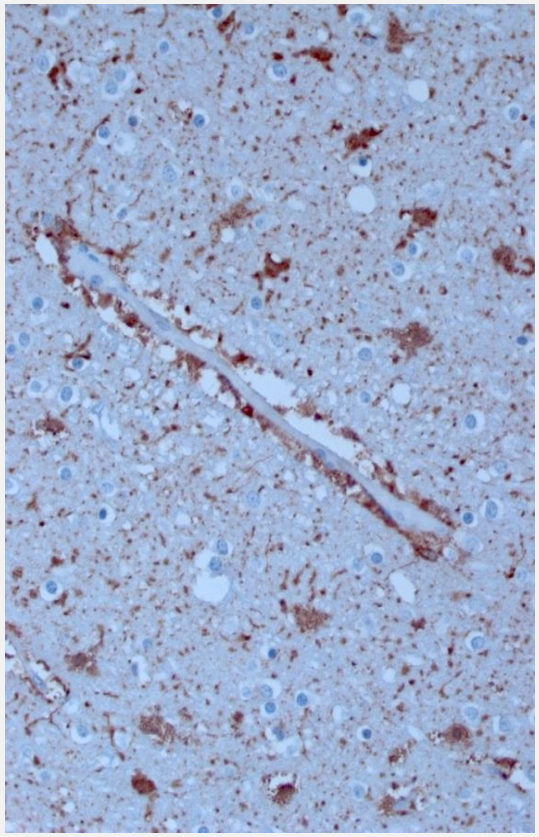


J Infect Dis. 2015;212(9):1459-1468. doi:10.1093/infdis/jiv232 (Holznagel , et al.)

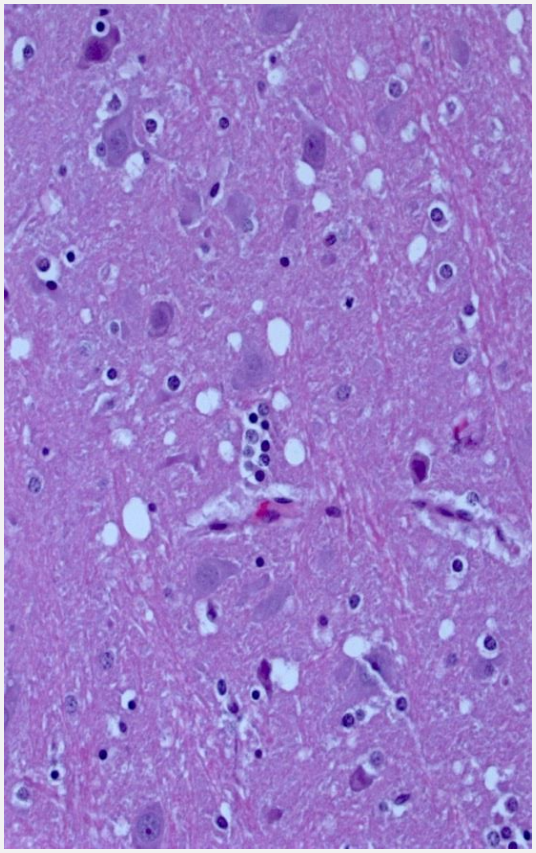
Lumbar spinal cord, H&E; 6C2, x100

Proof-of-concept: ic *sw; CWD WTD, 5.3 years (AU519)*

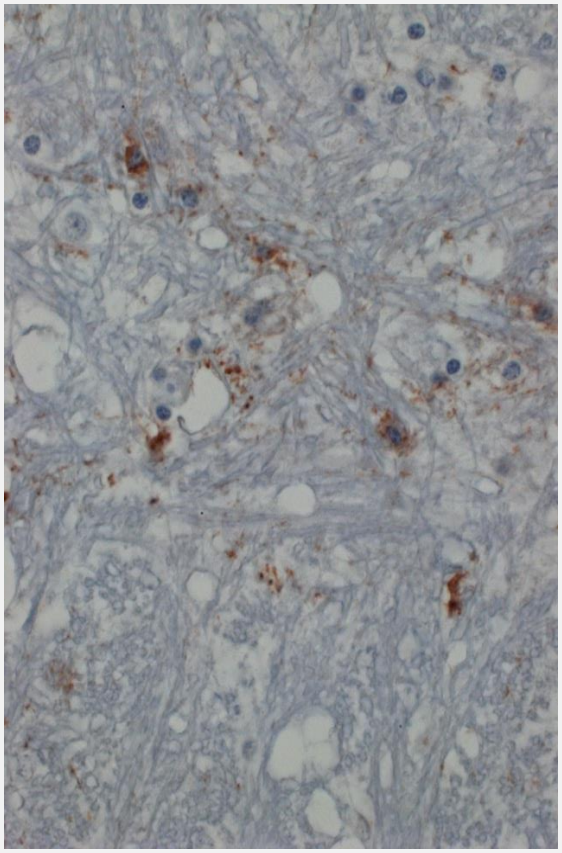
GFAP



H&E



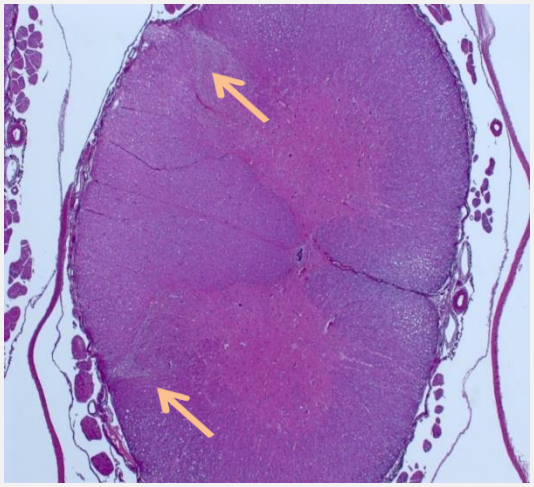
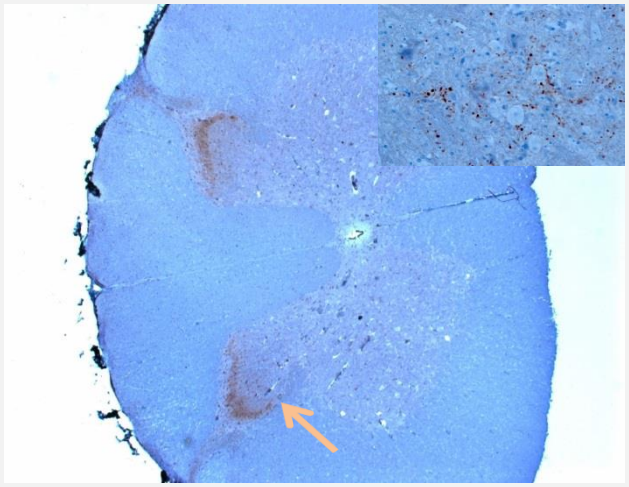
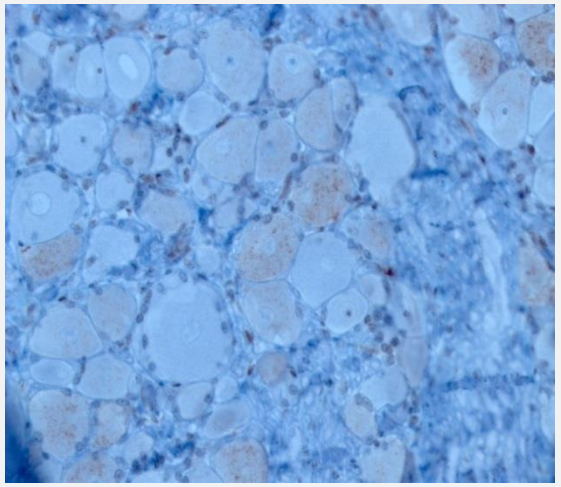
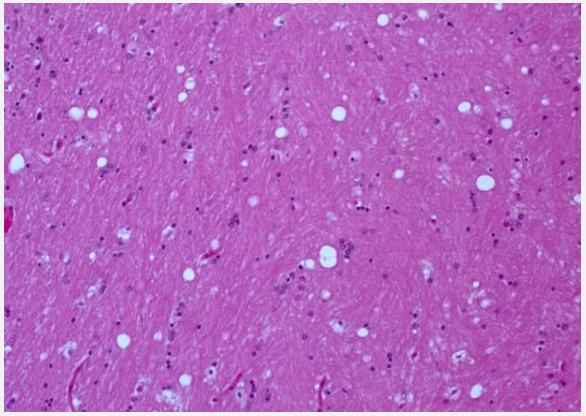
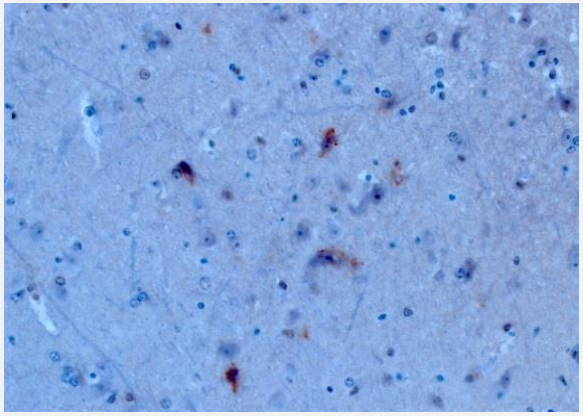
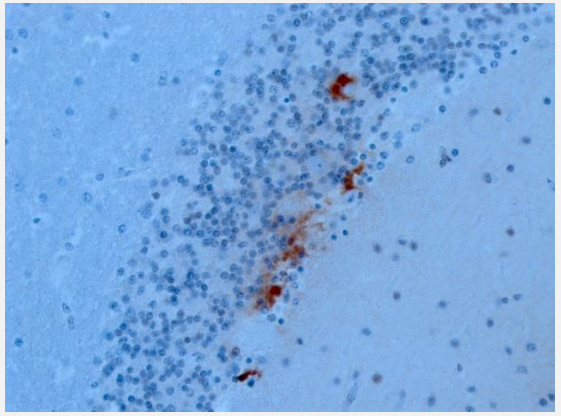
6H4



Obex, x200x (GFAP, H&E) | 400x (6H4)

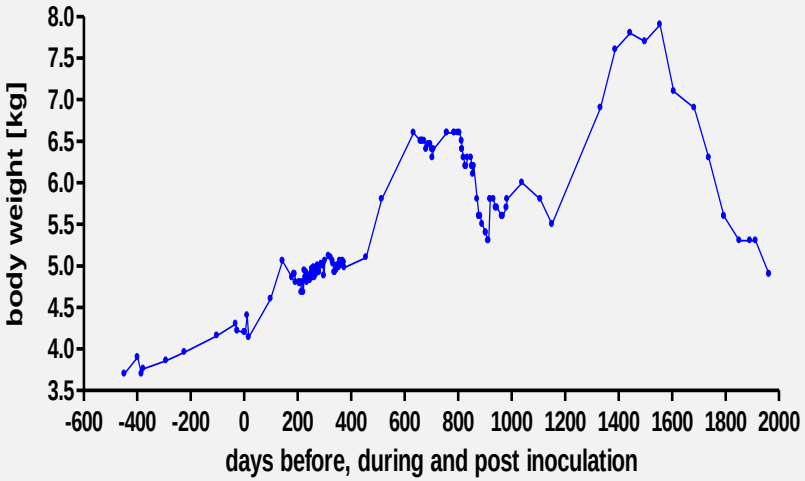
Per oral: *CWD WTD brain, 5.8 years (AU467)*

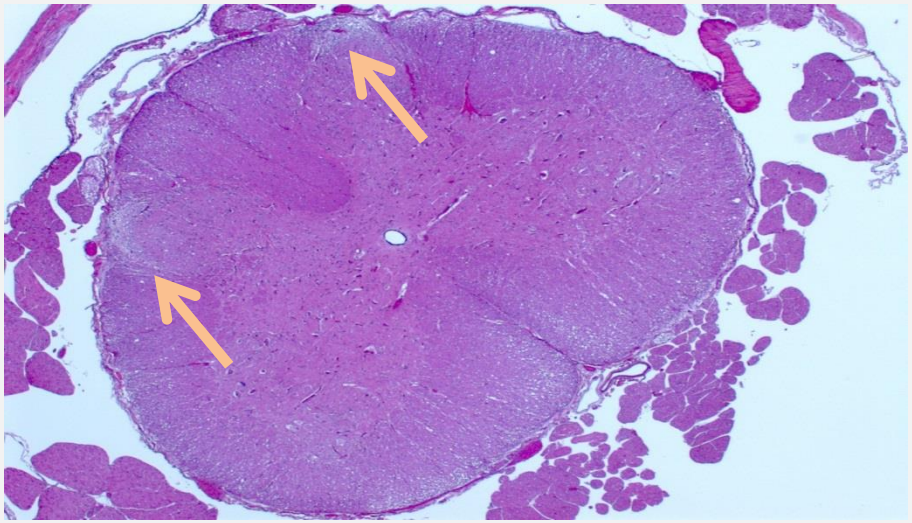
H&E **6H4** **6H4**

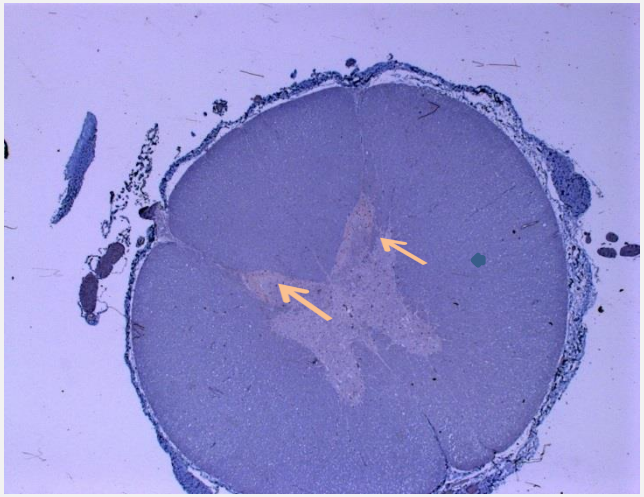







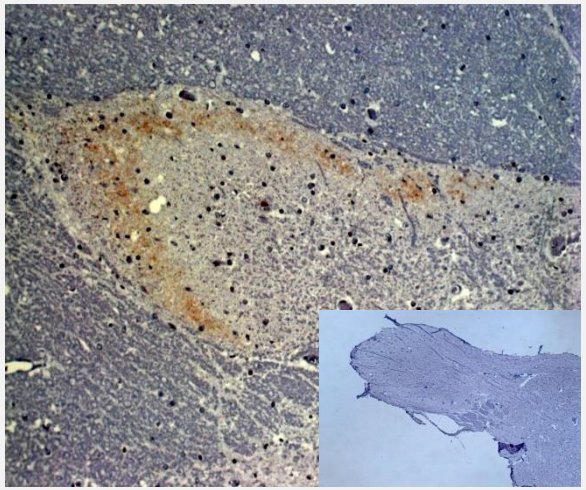
Lumbar Spinal Cord: x100 (H&E & 6H4)), x200 (6H4); DRG: x400 (6H4); Pons: x200 (H&E, 6H4); cerebellum x200 (6H4)

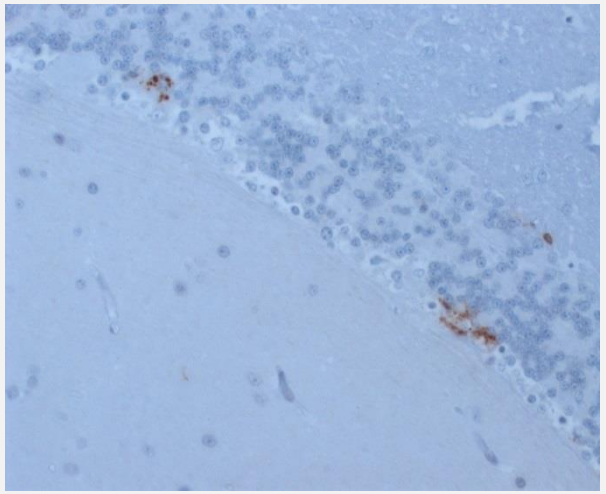
Per oral, *CWD WTD muscle, 5.4 years: Weight, H&E &IHC (AU501)*





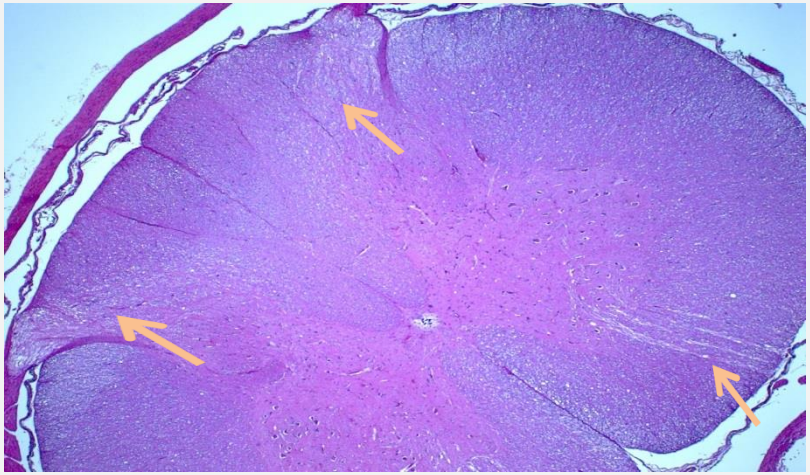


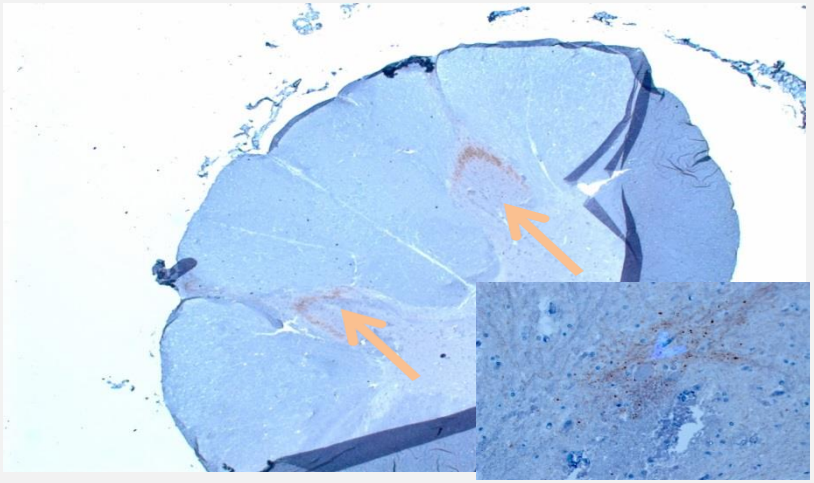


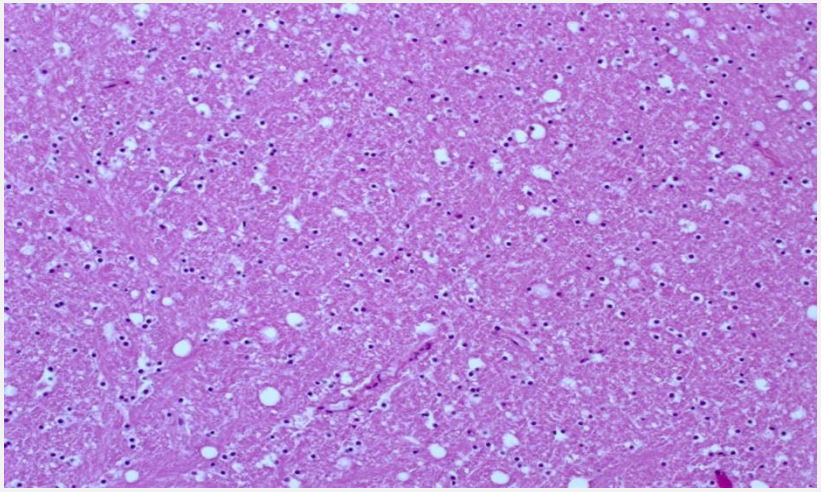


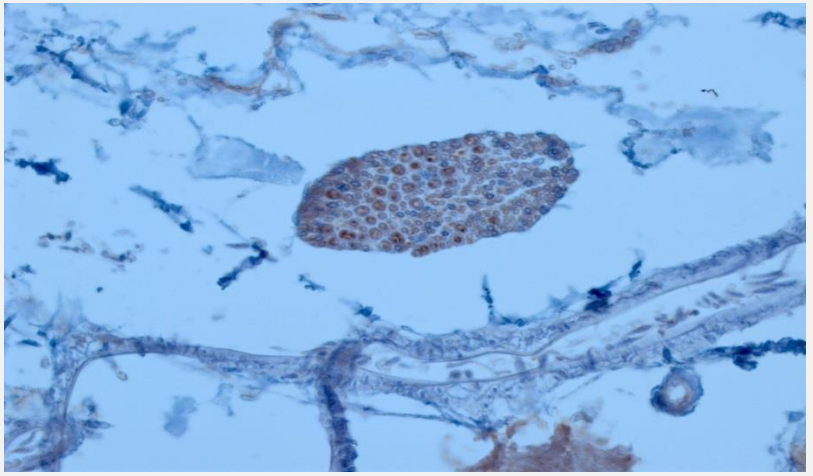
Lumbar Spinal Cord: x2.5 (H&E & 6H4)), x200 (6H4); cerebellum x200 (6H4)

Per oral: *CWD WTD muscle; 6.2 years (AU385)*









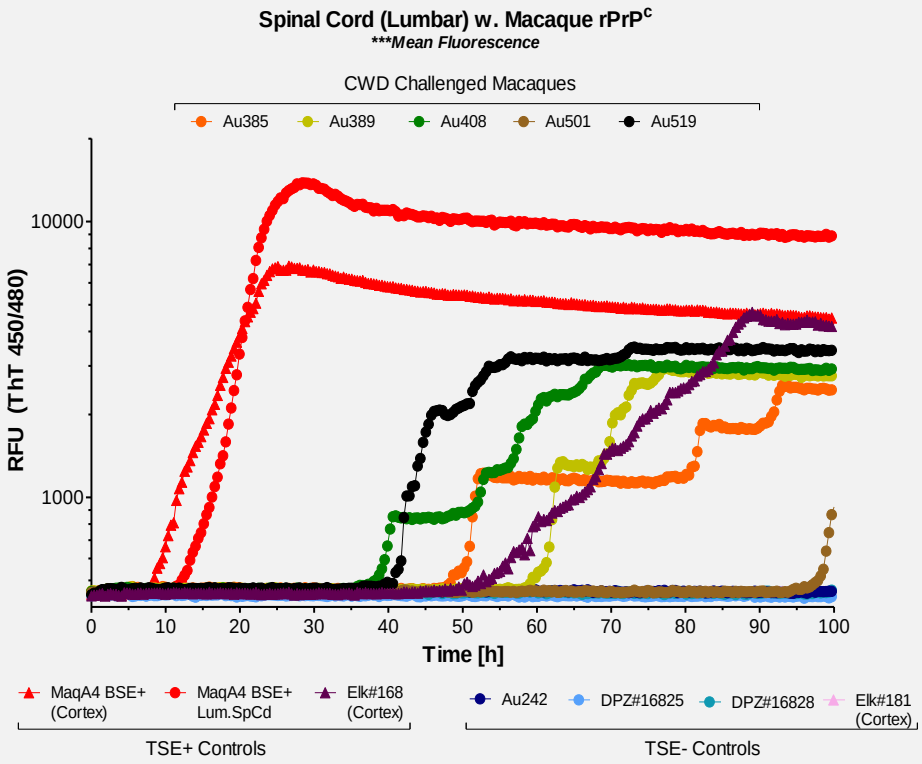
Spinal cord, H&E, x100, 6H4 IHC x25 & 200: Pons, H&E, x200; afferent nerve, 6H4, x200

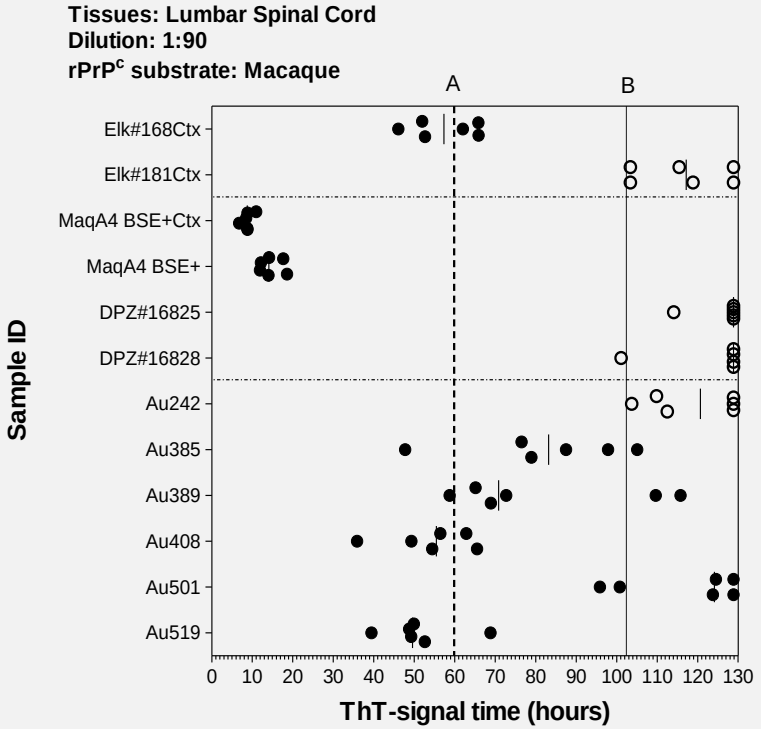
RT-QuIC

AMYLOID SEEDING ACTIVITY

RT-QuIC: lumbar spinal cord

(fluorescence curve & dot blot ROC characteristics)



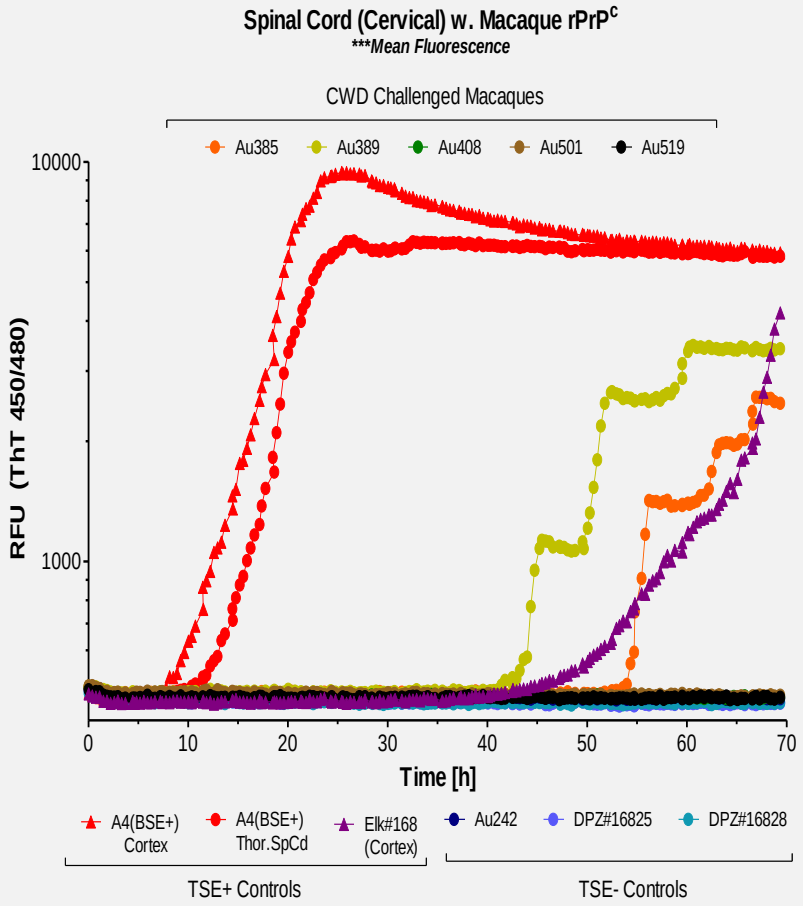


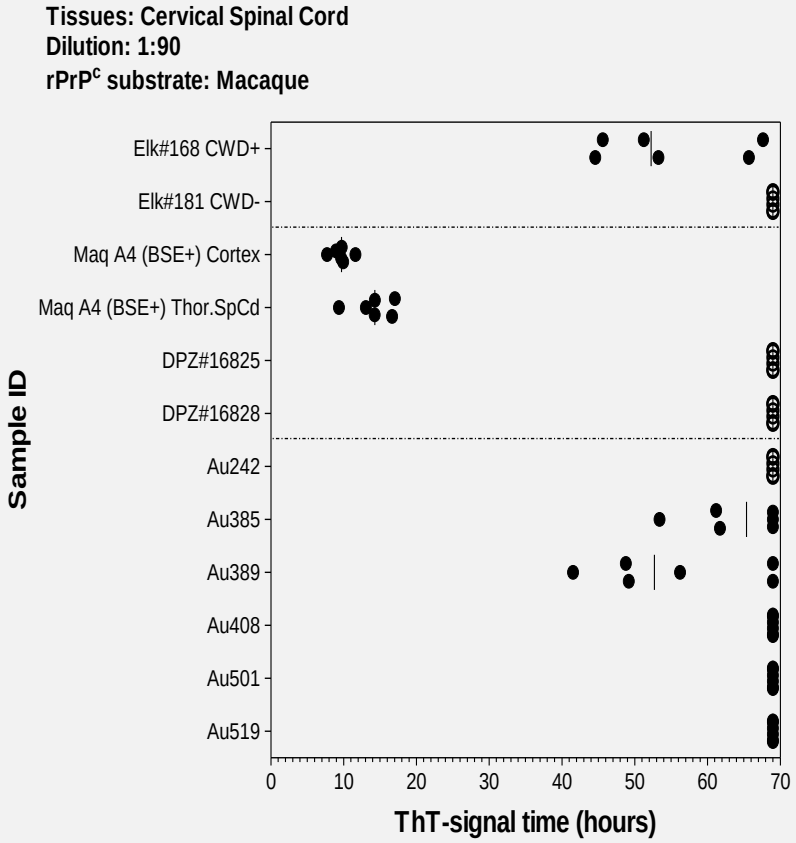
A: 100% Sens/Spec for BSE+ Macaque and DPZ/Au242 controls
B: 100% Sens., 94% Spec. for BSE+ Macaque and DPZ/Au242 controls

Gray, J. G., Graham, C., Dudas, S., Paxman, E., Vuong, B., & Czub, S. (2016). Defining and assessing analytical performance criteria for a TSE-detecting amyloid seeding assay. *Journal of Molecular Diagnostics, 18*(3), 454-467. doi: 10.1016/j.jmoldx.2016.01.005

RT-QuIC: cervical spinal cord

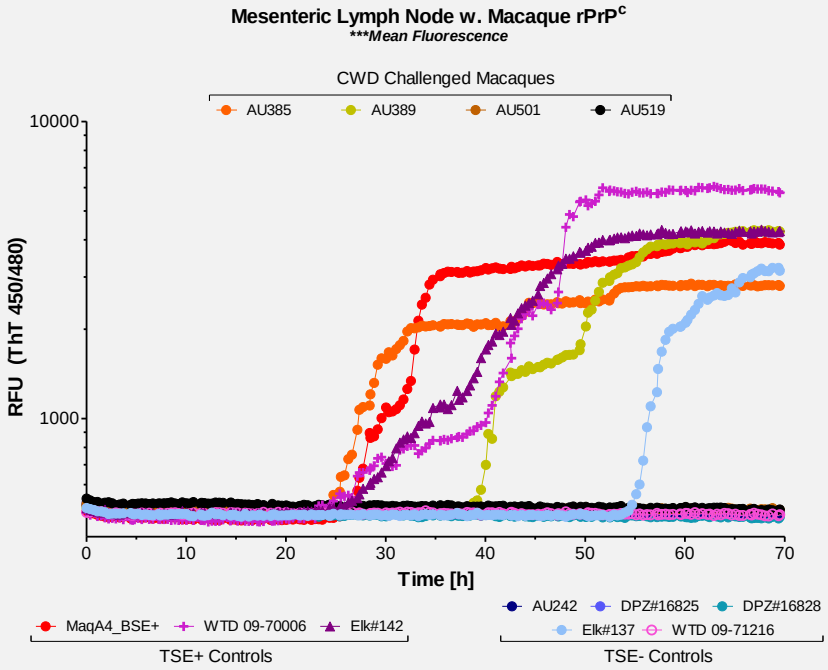
(fluorescence curve & dot blot ROC characteristics)

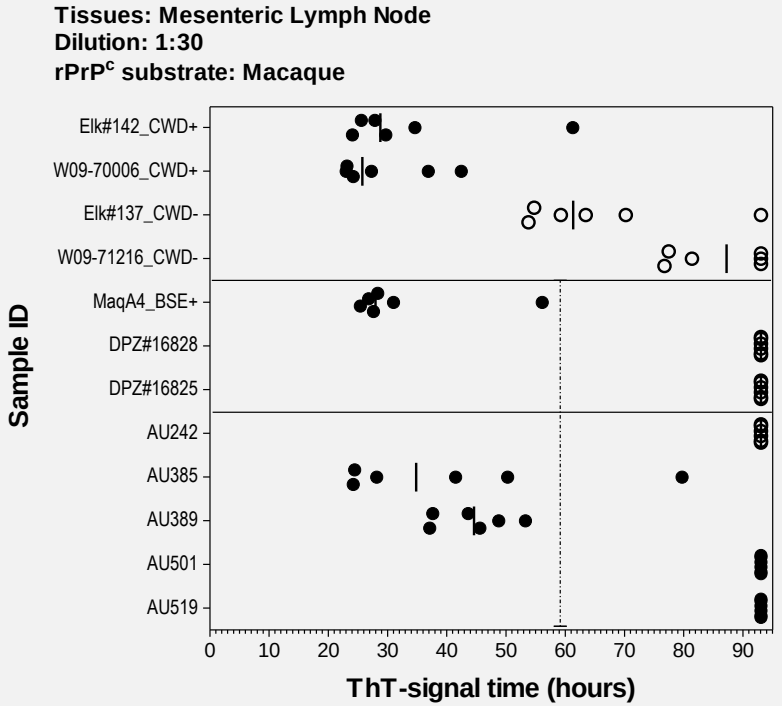




RT-QuIC: mesenteric lymphnode

(fluorescence curve & dot blot ROC characteristics)



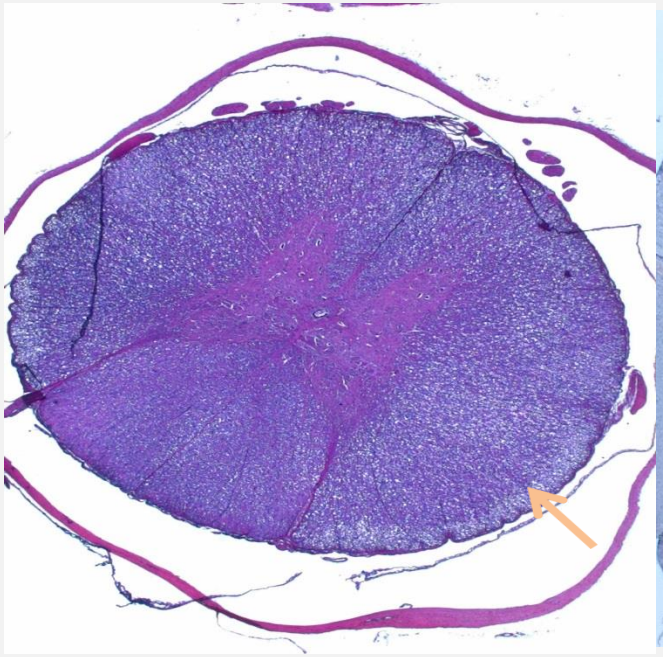


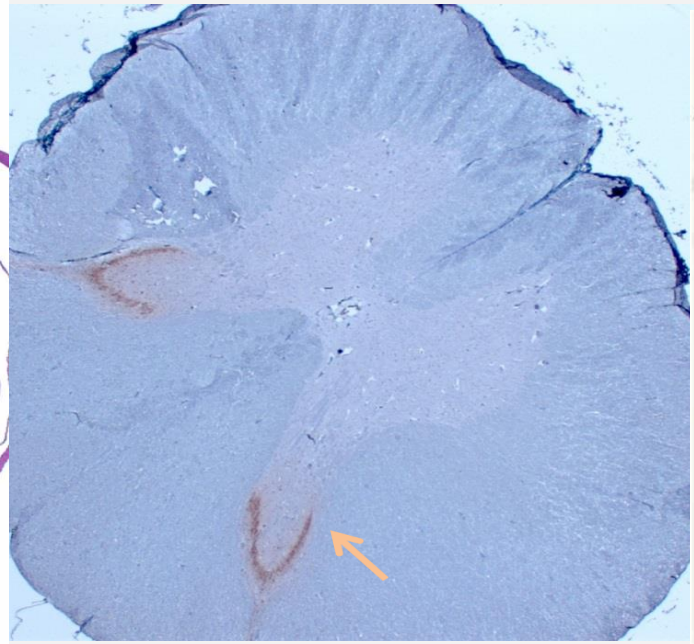
RESULTS

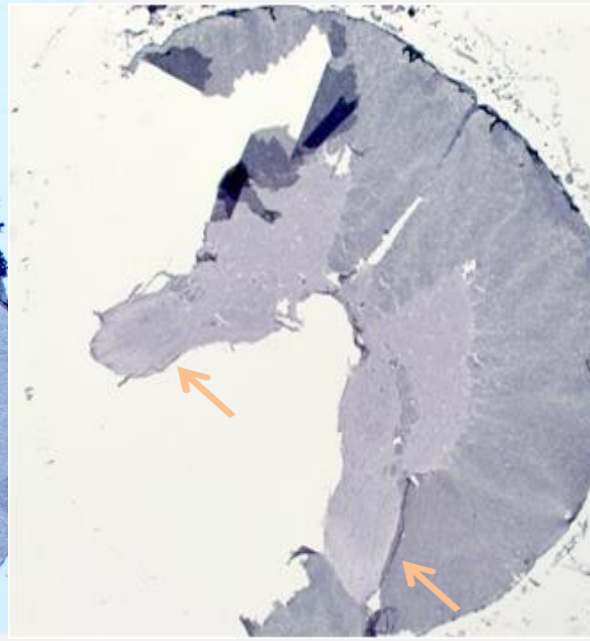
Results Summary

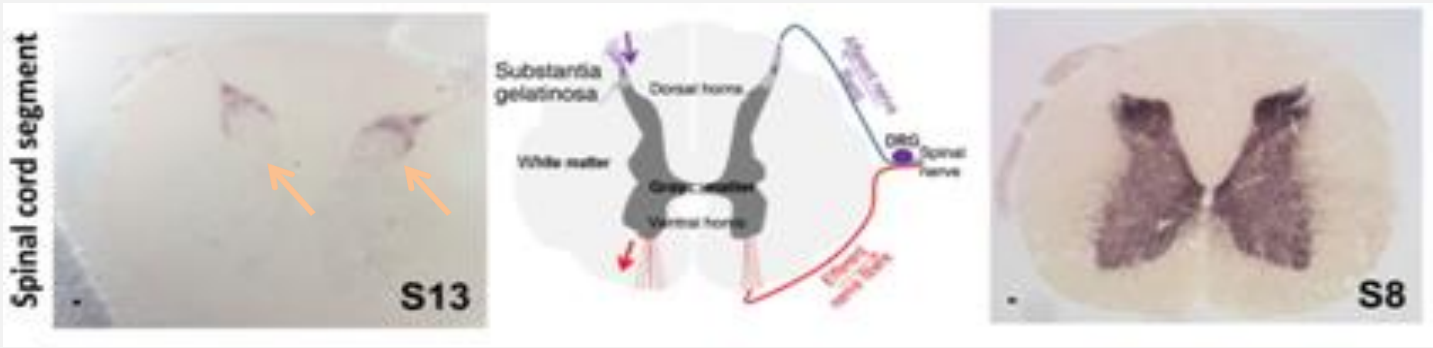
Animal #	AU389	AU519	AU467	AU501	AU385
Years p.c.	4.5	5.2	5.9	5.4	6.3
Route	i.c. steel wire	i.c. steel wire	oral	oral	oral
Inocula	Elk+	WTD+	WTD+ brain	WTD+ muscle	WTD+ muscle
Clinical disease	ataxia anxiety tremor wasting	scheduled PM	wasting (died p.anesth.)	ataxia anxiety tremor wasting	ataxia apathy tremor wasting
H&E/IHC	+ +	+ +	+ +	+ +	+ +
PMCA	ip	ip	ip	ip	ip
QuIC	+	+	+	+	+
Diabetes	+	-	-	-	+

Proof-of-concept: i.c. sw*; CWD elk, 4.5 years (AU389)*







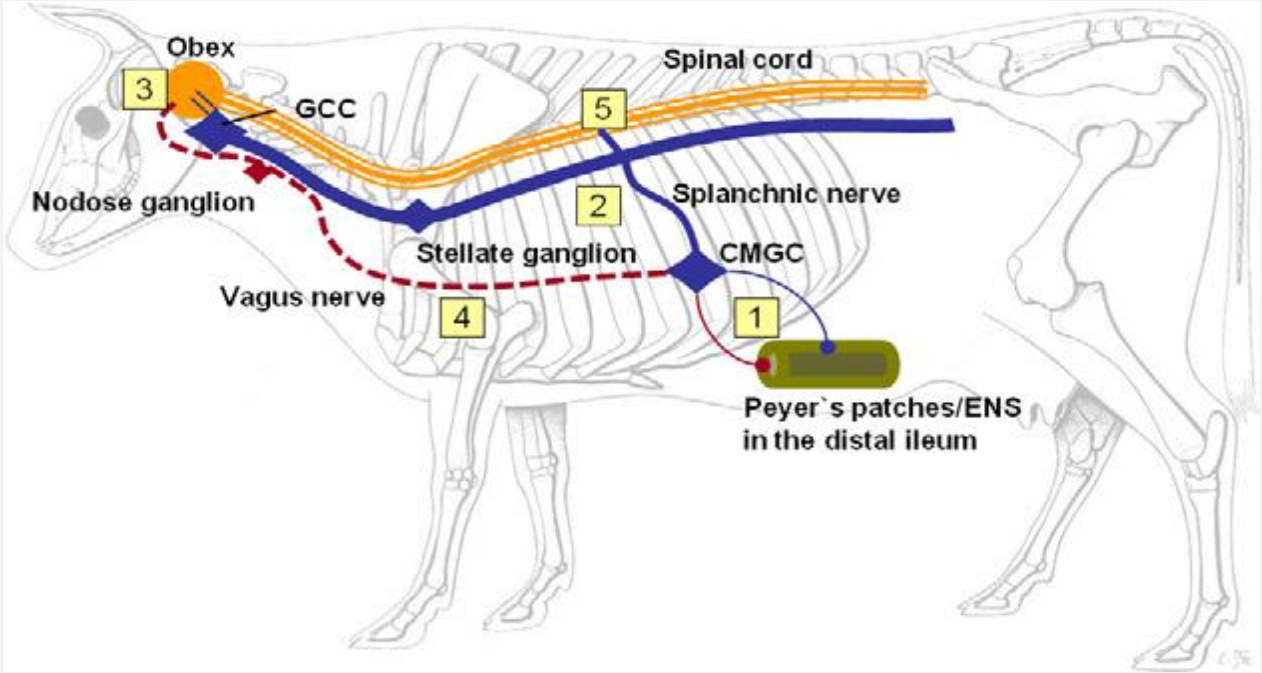


J Infect Dis. 2015;212(9):1459-1468. doi:10.1093/infdis/jiv232 (Holznagel , et al.)

Lumbar spinal cord, H&E; 6C2, x100

Spread & Distribution of C-type BSE *(Ch. Hoffmann et al, 2011)*

- 1) Peyer's patches
- 2) Sympathetic Ganglion Chain
- 3) Obex
- 4) Vagal nerve
- 5) Spinal cord



CWD transmission project: remaining macaques

Animal No.	Route of inoculation	Inoculum	Years post inoculation (07/2017)
AU500	i.c/ steel wire	CWD WTD pool	7.8
AU308	i.c/ steel wire	CWD elk	6.7
AU406	i.c.	mock control	8.0
AU398	skin scarification	CWD WTD pool	8.0
AU451	skin scarification	CWD WTD pool	8.0
AU315	oral	mock control	8.3
AU243	oral	CWD WTD brain pool	8.3
AU456	blood transfusion	plasma/buffy coat, RML elk	7.7
AU382	blood transfusion	plasma/buffy coat , RML WTD	7.7
AU390	blood transfusion	plasma/buffy coat , RML mule deer	7.7

CWD Macaque Transmission Project: in July 2017

- 11/21 animals available for assessment
- 10 remaining animals with no clinical signs (incubation: 4.2 – 7.9 years) (2 mock controls)
- 3/11 animals with neurological signs
- 6/11 animals with wasting (6/11 animals with confirmed pre-clinical or clinical diabetes)

So far:

- In 5 animals: prion specific histopathologic lesions; PrPsc deposits &/or amyloid seeding. *Read-out is ongoing (animal challenges; PET-BLOT; WB; PMCA)*
- 2 animals are i.c challenged (steel wire); 3 animals are challenged per orally with pre-clinical CWD WTD muscle (2/3) or clinical CWD WTD brain (1/3)
- Post mortems of remaining animals scheduled in 2018 (potentially in absence of clinical disease)

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Thank you for attending this webinar. If you have additional questions or comments concerning Chronic Wasting Disease or other prion diseases please email :

prion@cdc.gov

Slides and the recording for this presentation will be posted to the CSTE webinar library