Mysteries of prion diseases: transmission and biosafety

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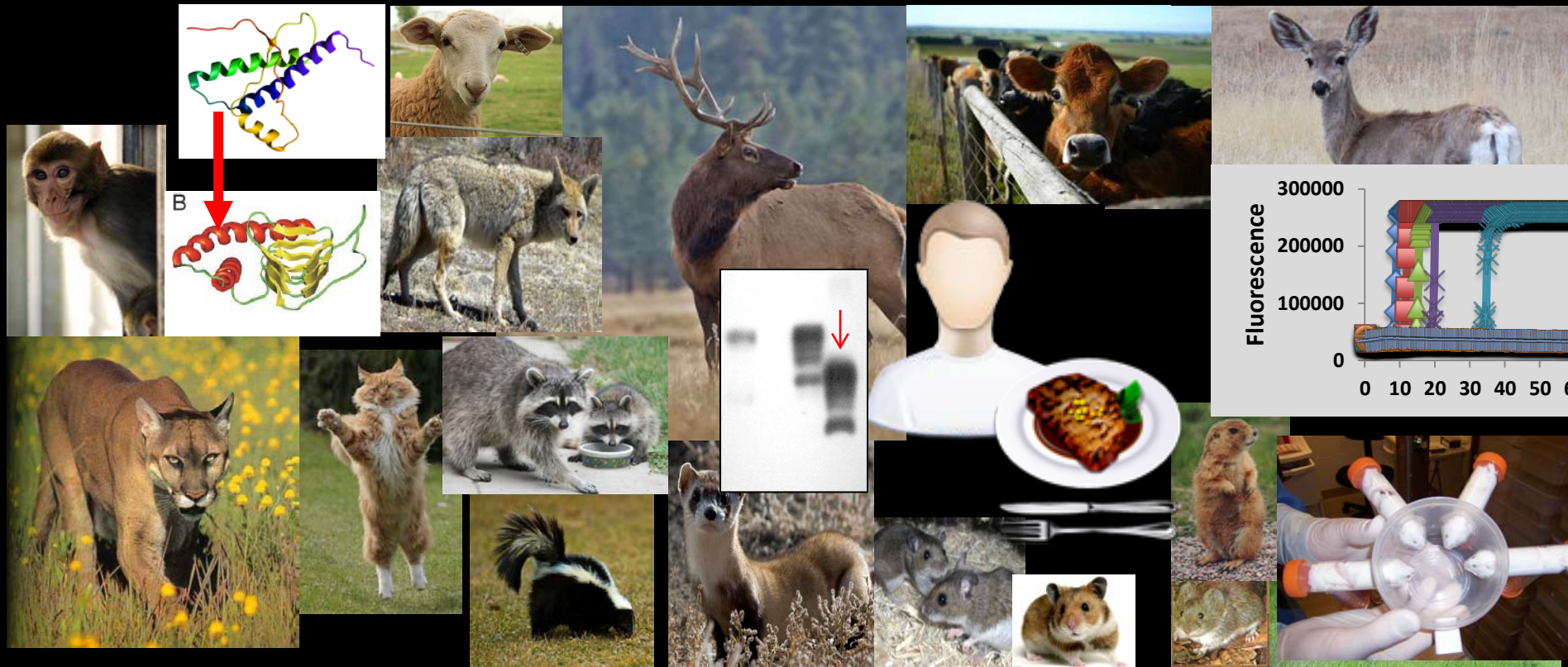

Colorado State University

College of Veterinary Medicine and Biomedical Sciences

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PrP

Prion Research Program

Prion Diseases [aka Transmissible Spongiform Encephalopathies (TSEs]

Scrapie

Bovine Spongiform Encephalopathy

Kuru

Creutzfeldt-Jakob Disease
Variant Creutzfeldt-Jakob Disease
Fatal Familial Insomnia
Gerstmann-Straussler-Scheinker Syndrome

Transmissible Mink Encephalopathy

Feline Spongiform Encephalopathy

Chronic Wasting Disease

Prion Diseases: aka Transmissible Spongiform Encephalopathies (TSEs):

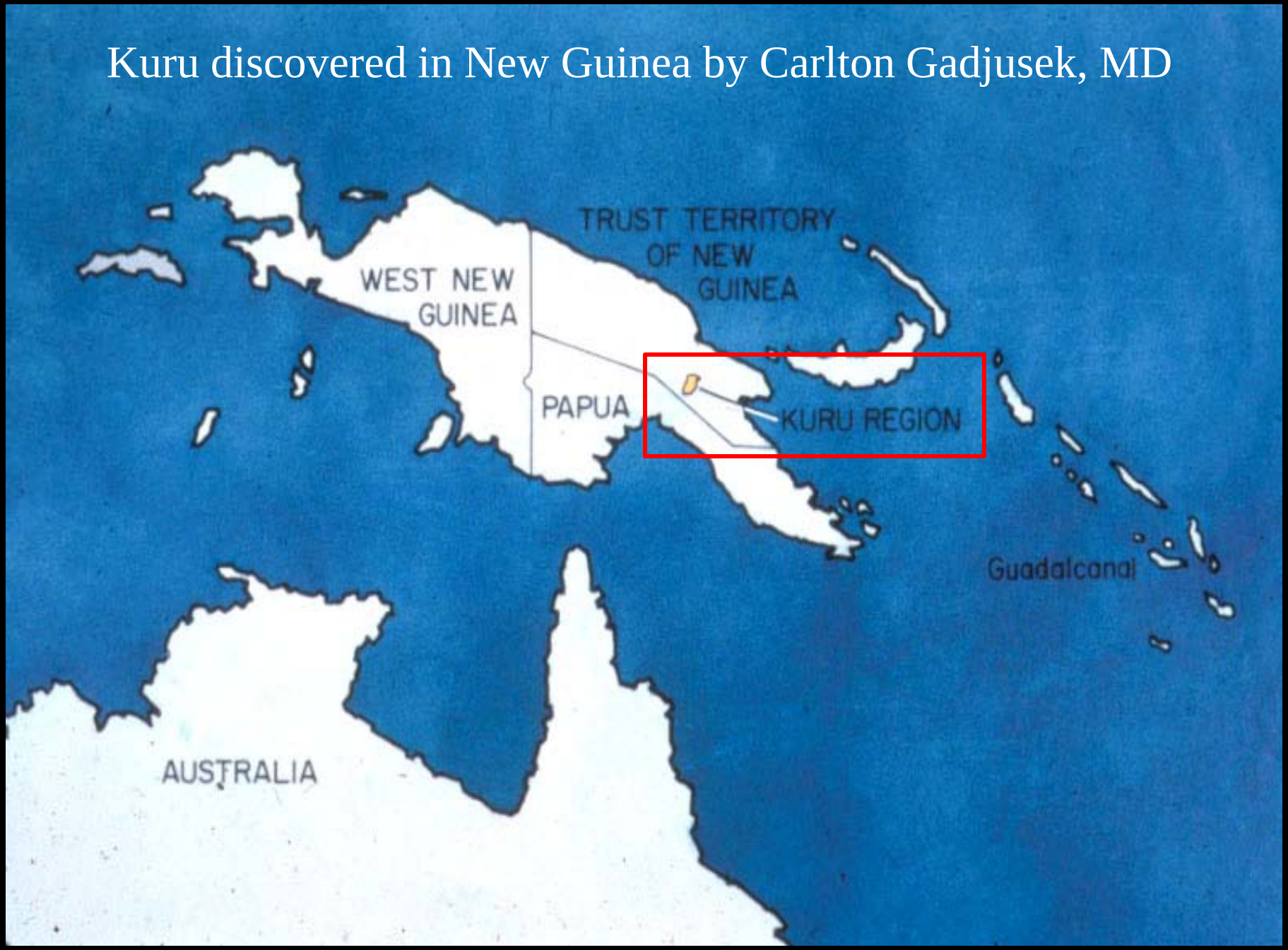
Animals:

- Scrapie of sheep
- Bovine spongiform encephalopathy (BSE)
- Feline spongiform encephalopathy (FSE)
- Transmissible Mink Encephalopathy (TME)
- Chronic wasting disease (CWD) of cervids

Humans:

- Creutzfeldt-Jakob Disease (CJD)
- Kuru
- Fatal Familial Insomnia (FFI)
- Gerstmann-Straussler-Scheinker Syndrome (GSSS
- Variant Creutzfeldt-Jakob Disease (vCJD)

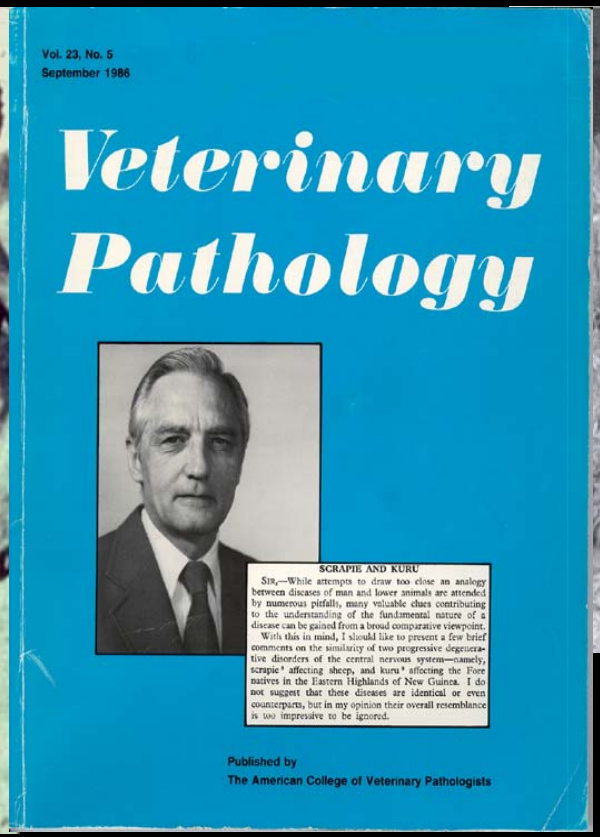
Kuru discovered in New Guinea by Carlton Gadjusek, MD



The connection of Kuru to scrapie as TSEs made by William Hadlow, DVM

Kuru

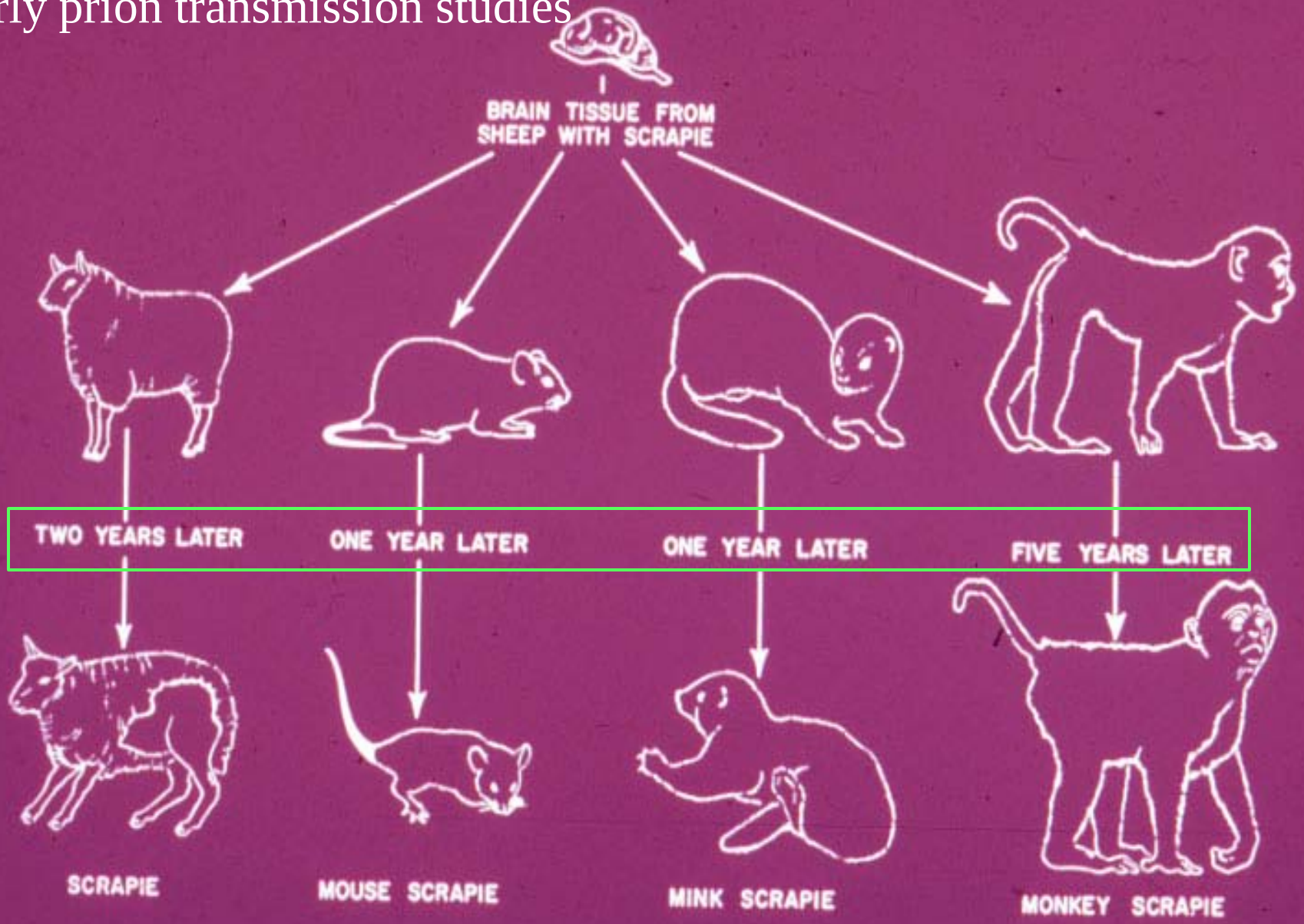
by C. Gadjusek



Scrapie

by G.A. Wells and S.A.C. Hawkins

Early prion transmission studies



Very long incubation periods, but amazingly, cross species transmission

Prion protein: normal protein and proteinacous infectious particle

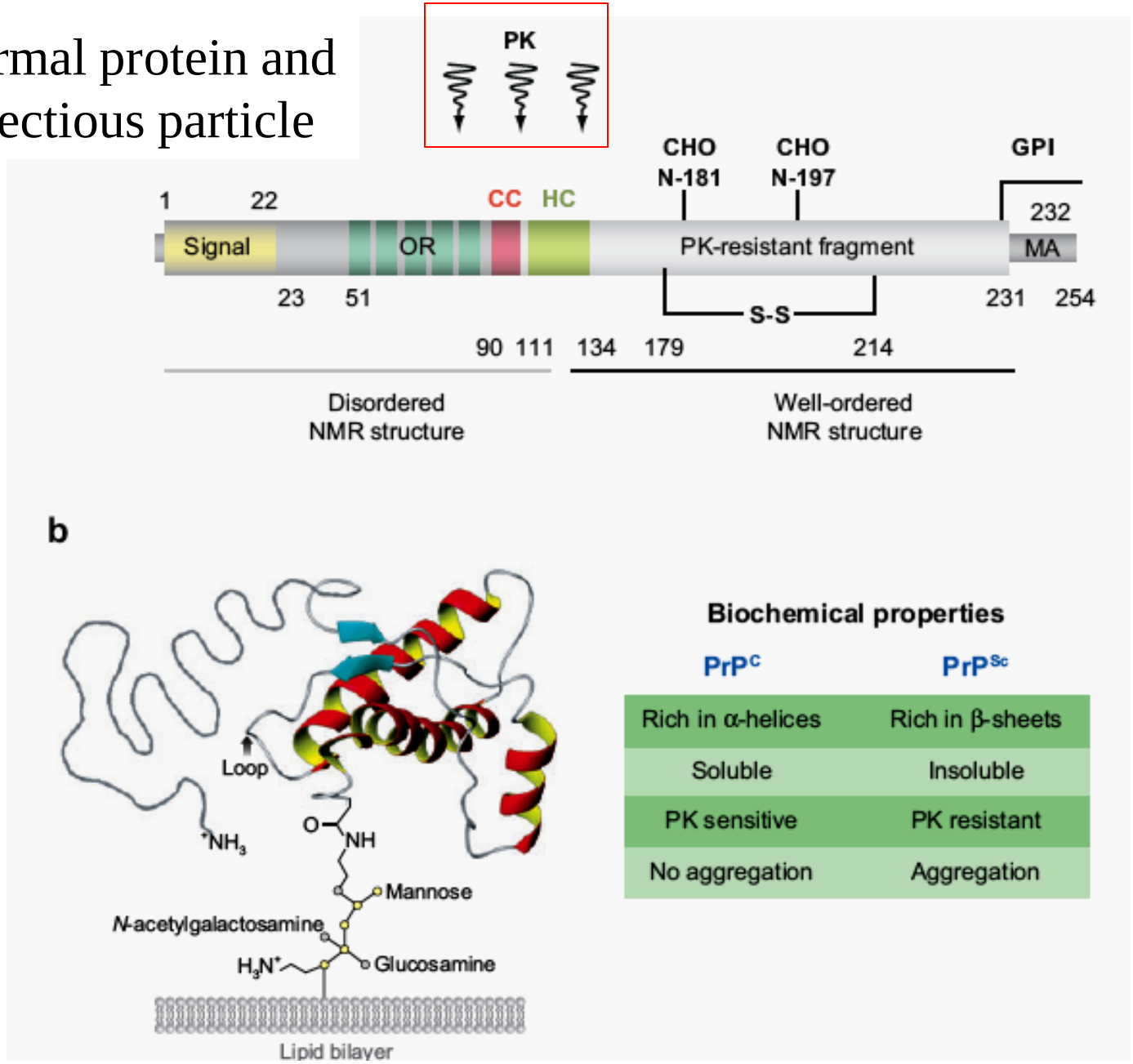
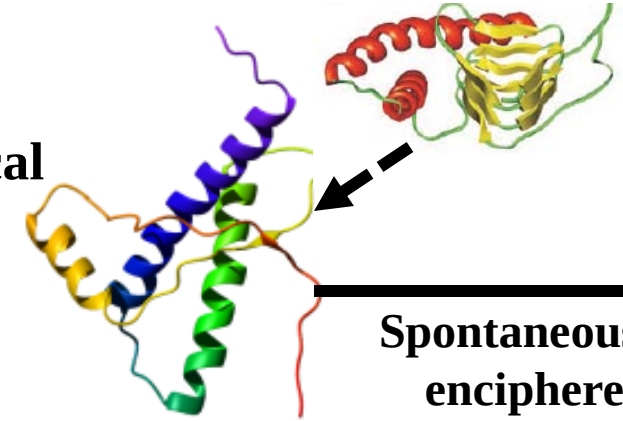


Figure 2 (*a*) Scheme of the primary structure of the cellular prion protein and its posttranslational modifications. A secretory signal peptide resides at the extreme N terminus. The numbers describe the positions of the respective amino acids. The proteinase K (PK)-resistant core of PrPSc is depicted in gray; the approximate cutting site of PK within PrPSc is indicated by arrows. CC (*pink*), charged cluster; HC (*green*), hydrophobic core; S-S, single disulfide bridge; MA, membrane anchor region; GPI, glycosyl phosphatidyl inositol; CHO, facultative glycosylation sites; NMR nuclear magnetic resonance.

Aguzzi A Heikenwalder M Molecular Mechanisms of Prion Pathogenesis Ann Rev Path 2008

Prion conversion (more B movie vs. religious)



- Soluble
- Alpha helical
- GPI-linked
- Protease-sensitive
- Highly conserved
- 253-6 aa
- Function still uncertain

PrP^{C}

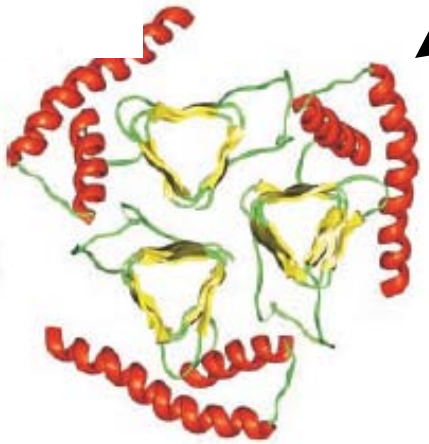
Good

Spontaneous or enciphered

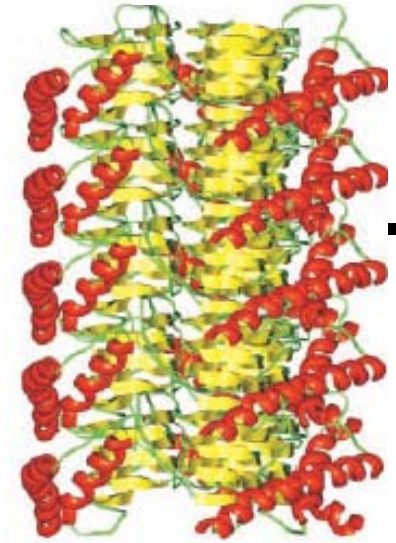
PrP^{RES}

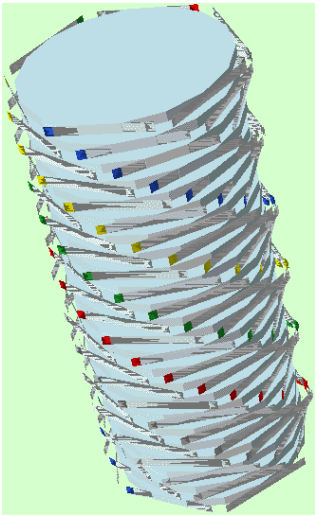
Evil

- Insoluble
- High ß-sheet content
- Protease-resistant
- Cofactor molecules in conversion still elusive



PrP^{RES} oligomer





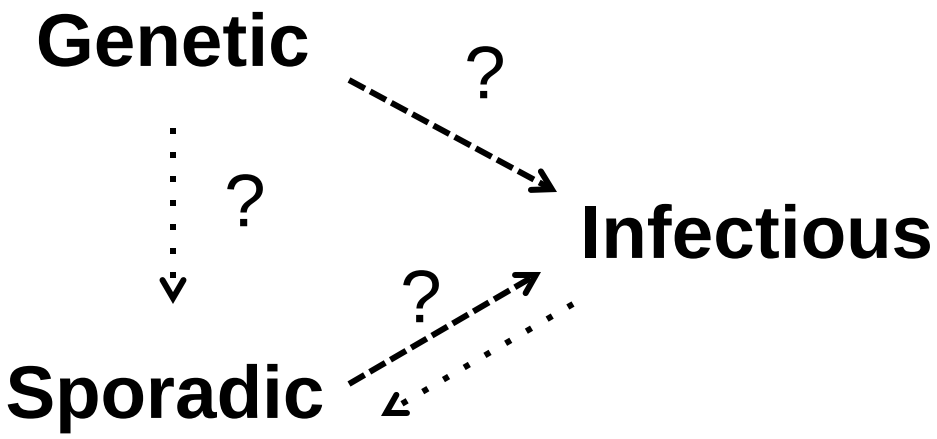
PrP^{RES} fibrils w beta sheet core

Discovery of prions: the subject of two Nobel prizes: C. Gadjusek and S. Prusiner

1976

1997

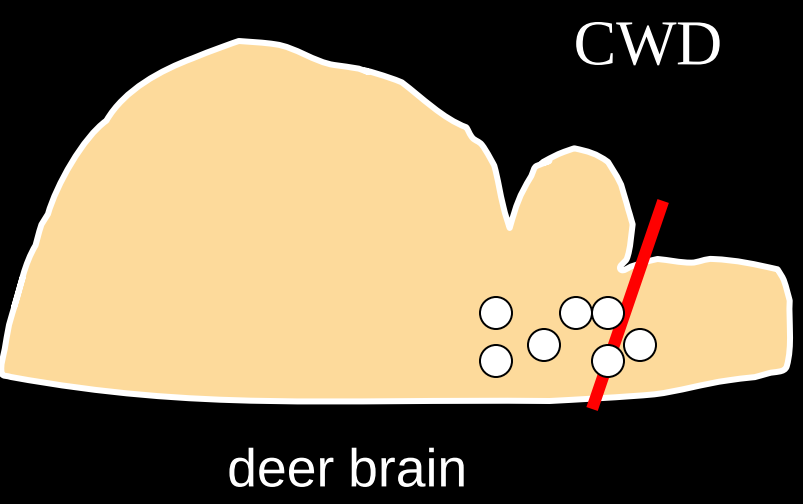
Prion diseases:
genetic? sporadic? infectious?
….yes

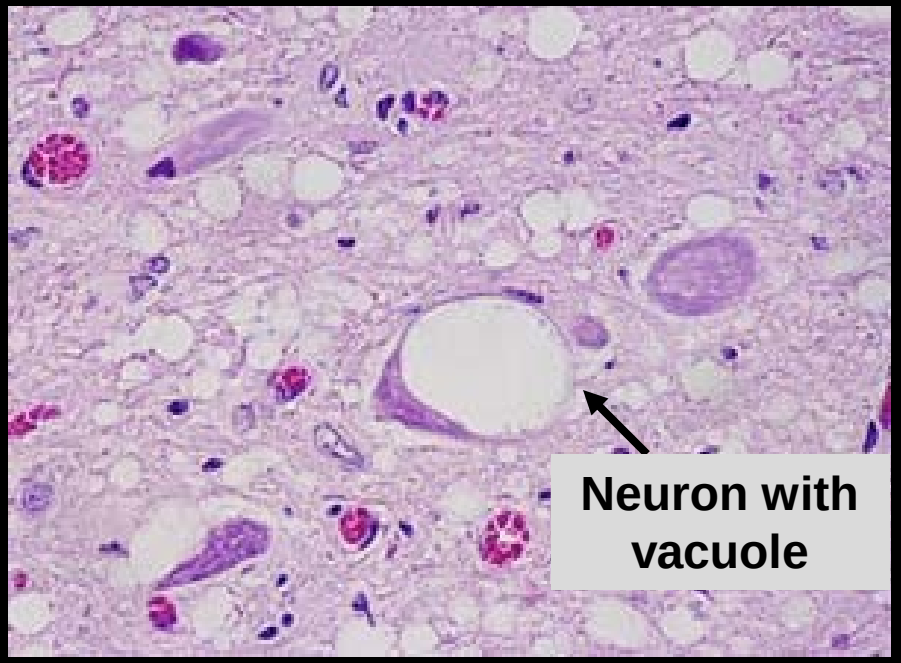


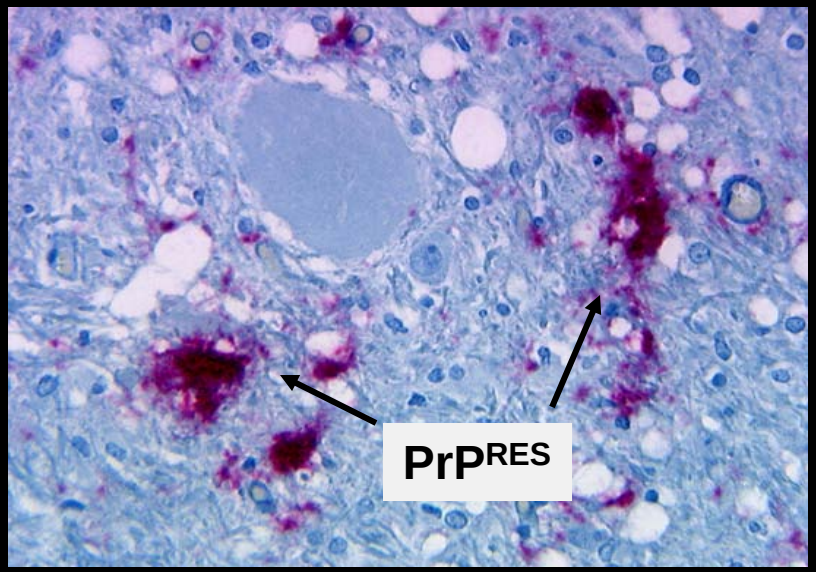
Transmission of prion diseases

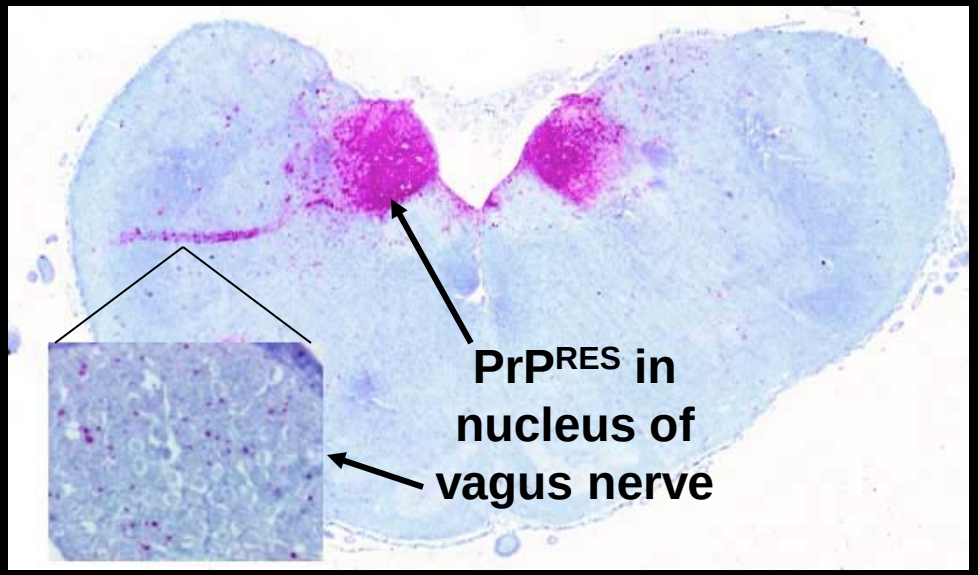
- **Kuru: ritualistic cannibalism**
- **sCJD: iatrogenic, tissue grafts, and unknown**
- **BSE, FSE, TME, vCJD: ingestion of prion-contaminated animal products**
- **CWD, scrapie: horizontal -- saliva, urine, feces, environment**
- **….but origin of the initial prions assumed to reflect spontaneous misfolding events**

TSE's: spongiform brain lesions associated with PrP^{RES}





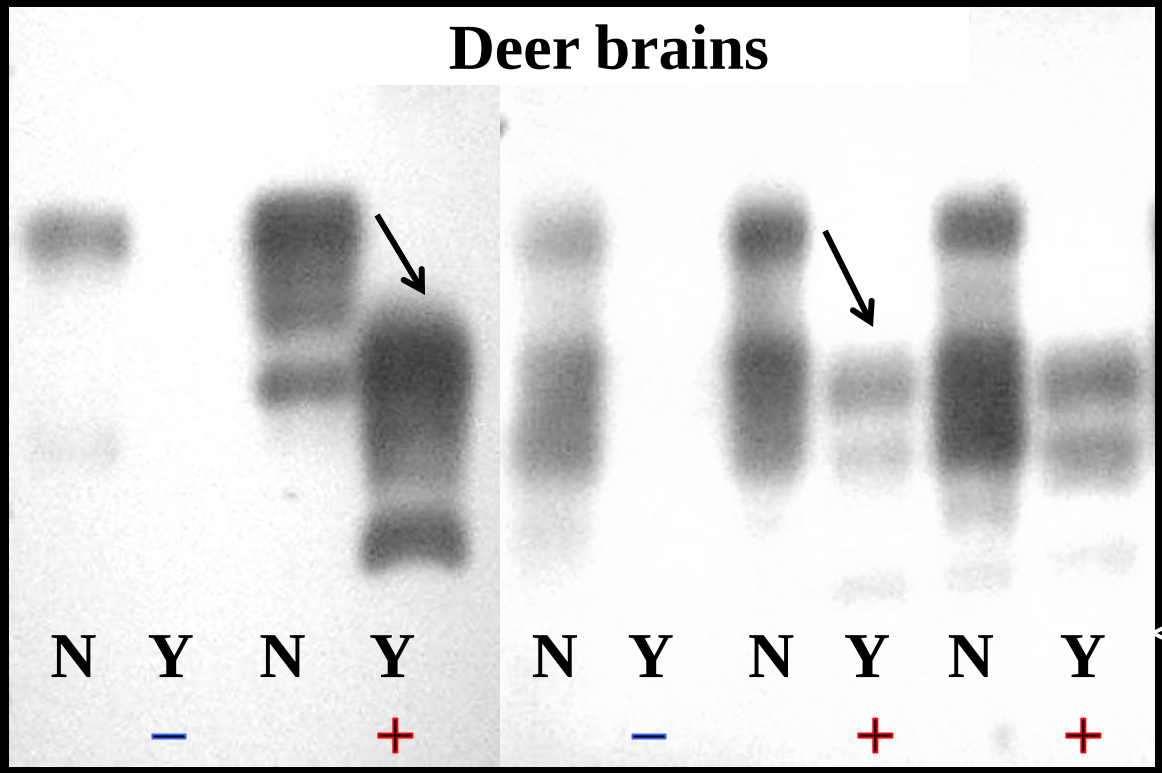




Prion disease diagnosis
Prion detection

- **Clinical signs, CT & MRI patterns**
- **Detection of protease resistant PrP (PrP^{RES})**
  - **Western blot**
  - **Immunohistochemistry**
  - **ELISA**
- **Detection of infectious prions:**
  - **Bioassay in animals**
    - **Mice, or more recently, PrP transgenic mice**
  - **Evolving in vitro assays**
    - **Seeding (PrP conversion)**
    - **Cell culture infection**

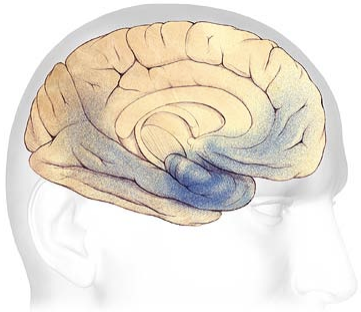
Detection of PK-resistant PrP^{RES} by western blotting



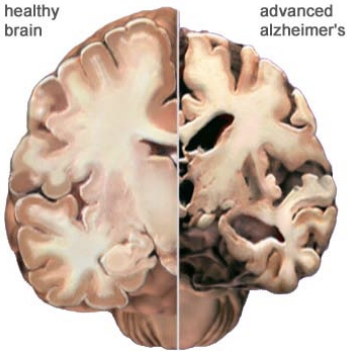
<- Protease digestion

<- CWD+?

Prion diseases and the major human neuro-degenerative diseases are linked by protein misfolding as a central process:



- **Alzheimer's disease (AD) – Abeta, Tau**
- **Parkinson's disease (PD) – Alpha synuclein**
- **Amyotrophic lateral sclerosis (ALS) – SOD1, TDP43**
- **Fronto-temporal dementia/head trauma -- TPD43, Tau**



All are characterized by polymerized mis-folded fibrillar protein deposits in the brain

The mechanism of protein mis-folding is likely similar to that in prion diseases

Prion biohazards: exposure

- **Contact transmission – no, not for human prions (we think)**

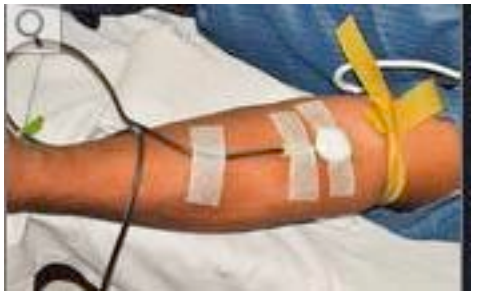
- **Food, supplements – yes (BSE)**

- **Blood, tissue transplnt. – yes (sCJD, vCJD)**

- **Surgical instruments – yes (vCJD)**

- **Environment – ?low for humans, but yes for animals due to persistence**

Prion biohazards: blood transfusion



- **In humans shown only for vCJD (BSE), not for sCJD. Preclinical donors.**
- **In animals, shown for deer and sheep. As little as 200µl of whole blood (scrapie)**
- **Prions are mostly WBC- and platelet-associated – leucodepletion used for all transfusions in UK**
- **Estimated in UK 1/2000 people *may* be carrying vCJD***

***UK Blood Transfusion & Tissue Transplantation Services**

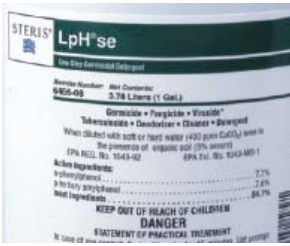
Prion infectivity and biosafety

- Not inactivated by conventional agents—formalin, alcohols, autoclaving at 122C
- Adhere to and persist on most surfaces, esp. metals, clay, silica, plastic
- Can persist in environment -- years
- Aerosolization? Not intensively studied thus far
- Minimum mucosal and IV infectious dose is unclear
- Species barrier occasionally breached

Prion biosafety: inactivation

- 1N NaOH
- 20,000 ppm)(2%) NaOCl
- Autoclaving at 134C

- 1% SDS + 0.1%HAc or NaOH (≥0.3%), 5-15 min. (i.e. SDS + high or low pH)
- Adding liquid phase autoclaving at 121C to the above



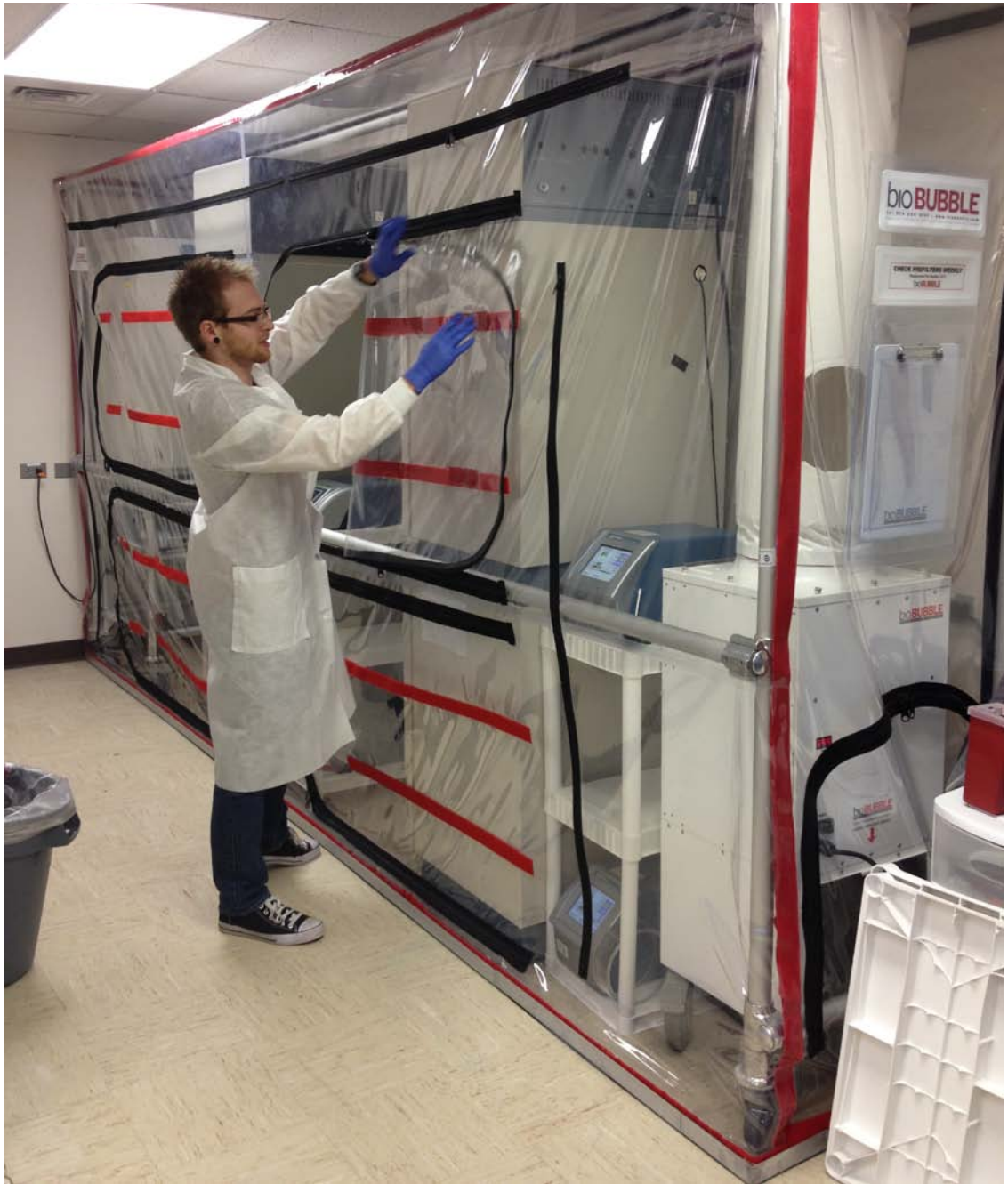
- Strong phenolic disinfectants (toxicity) or vaporized H_2O_2 reduce titer 4-5 logs
- Steel wires contaminated with 263K hamster scrapie is the most standard assay system

Prion biosafety: the species barrier

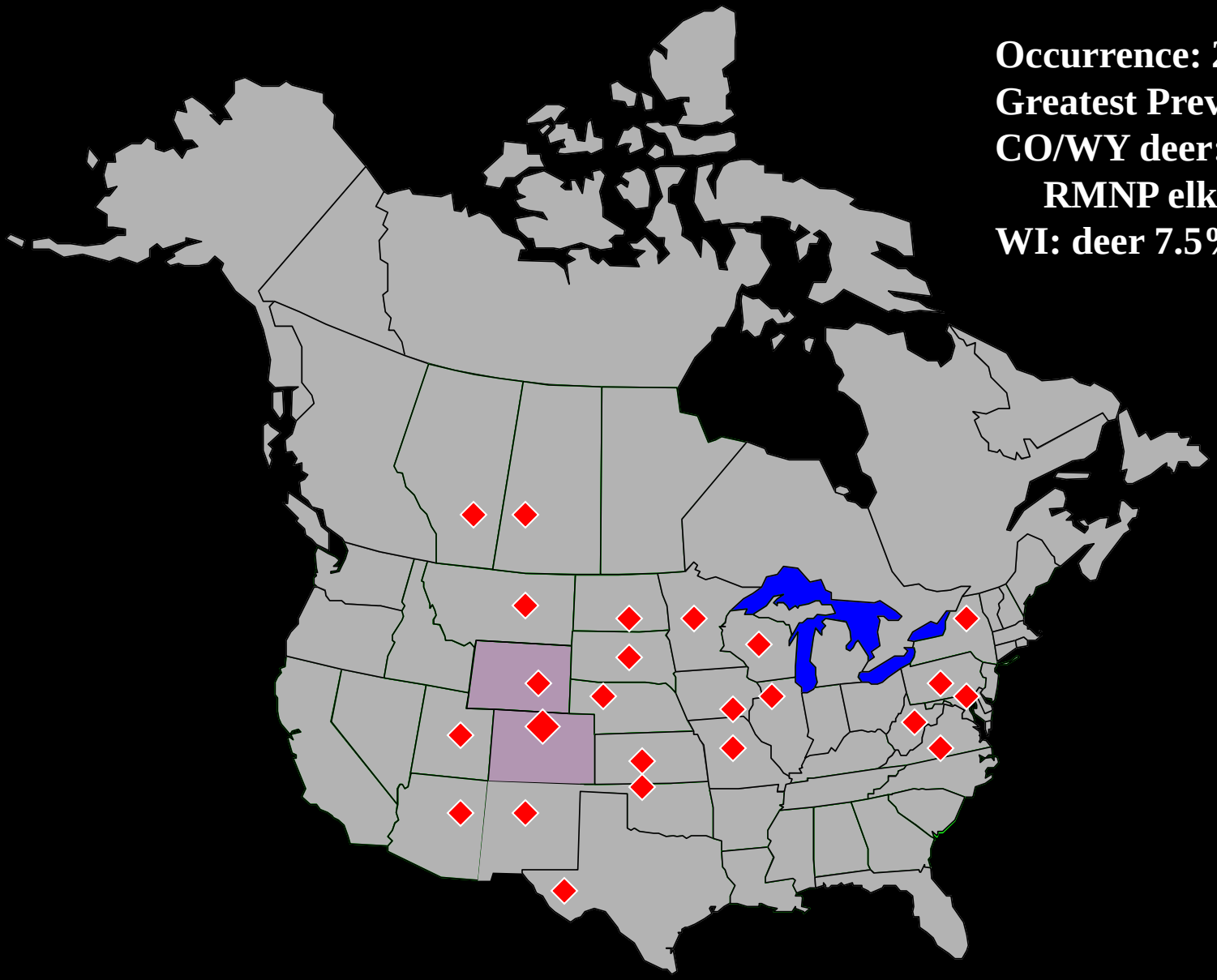
- **Like viruses, prions are usually species specific**
- **PrP^{C}<->PrP^{RES} sequence homology closely related to susceptibility**
  - **Transgenic expression of heterologous species PrP^{C} confers susceptibility of that species to that prion**
- **Species crossing demonstrated experimentally**
- **Indications of species barrier strength :**
  - **Incubation time (long vs. short) and attack rate (low vs. high)**
  - **Oral/nasal (natural) route susceptibility**
- **Still, species jumping occurs in nature—likely rarely and unpredictably**

Prion biosafety in lab: BSL2 (++)

BioBubble™ laminar flow containment of equipment and reagents for cross-species amplification or any BSE-related seeds. Closed transfer to biosafety cabinets



Chronic wasting disease: a prion disease of cervids

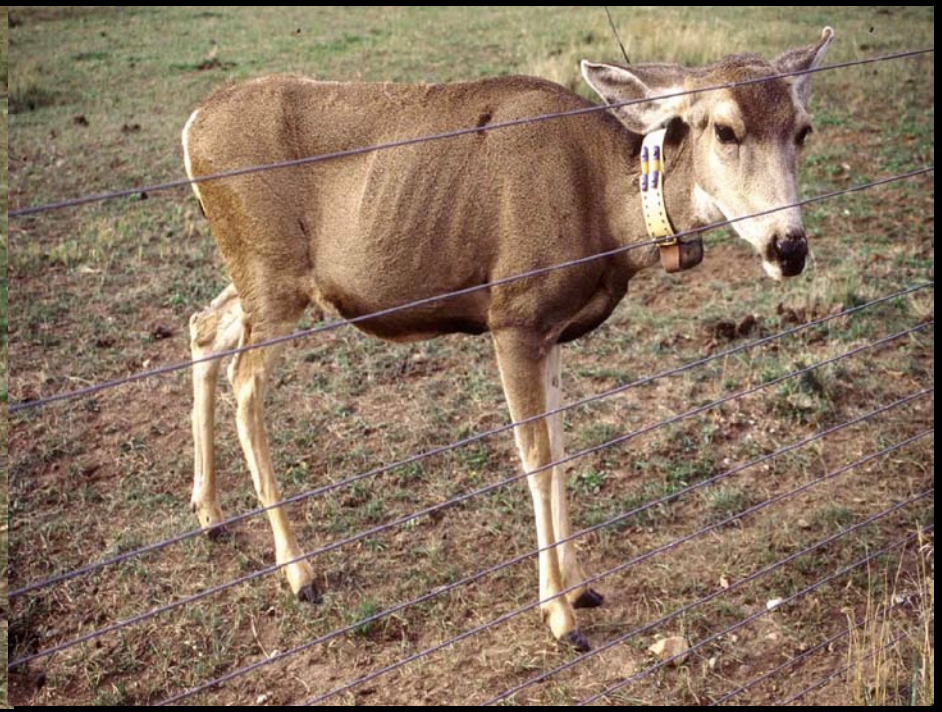


Occurrence: 22 States
Greatest Prevalence:
CO/WY deer: 5-10%
RMNP elk: 11-15%
WI: deer 7.5%

CWD Clinical disease

CWD negative mule deer

CWD positive mule deer

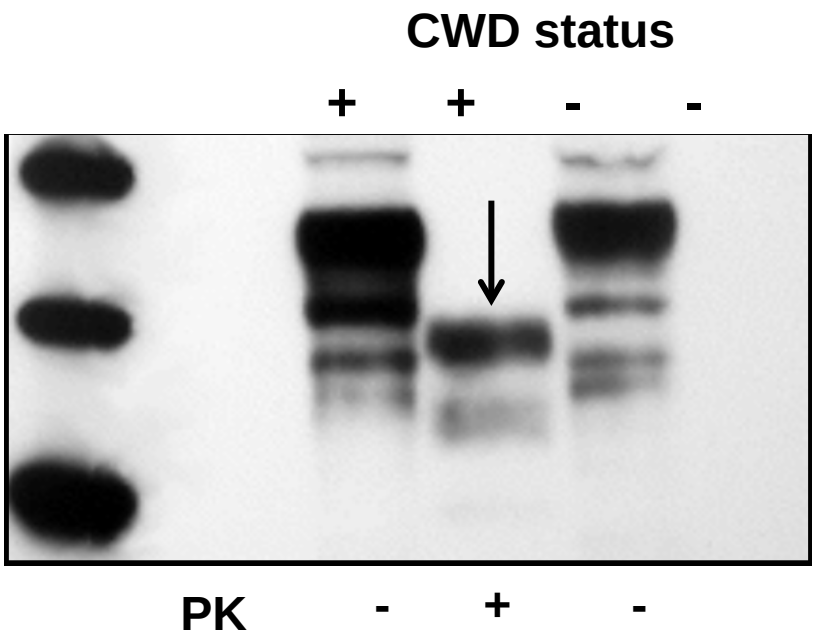


Behavioral changes, weight loss, ear, head position, gait change, stereotypic behaviors, polydipsia, polyphagia, staring, incoordination, digestive dysfunction, salivation

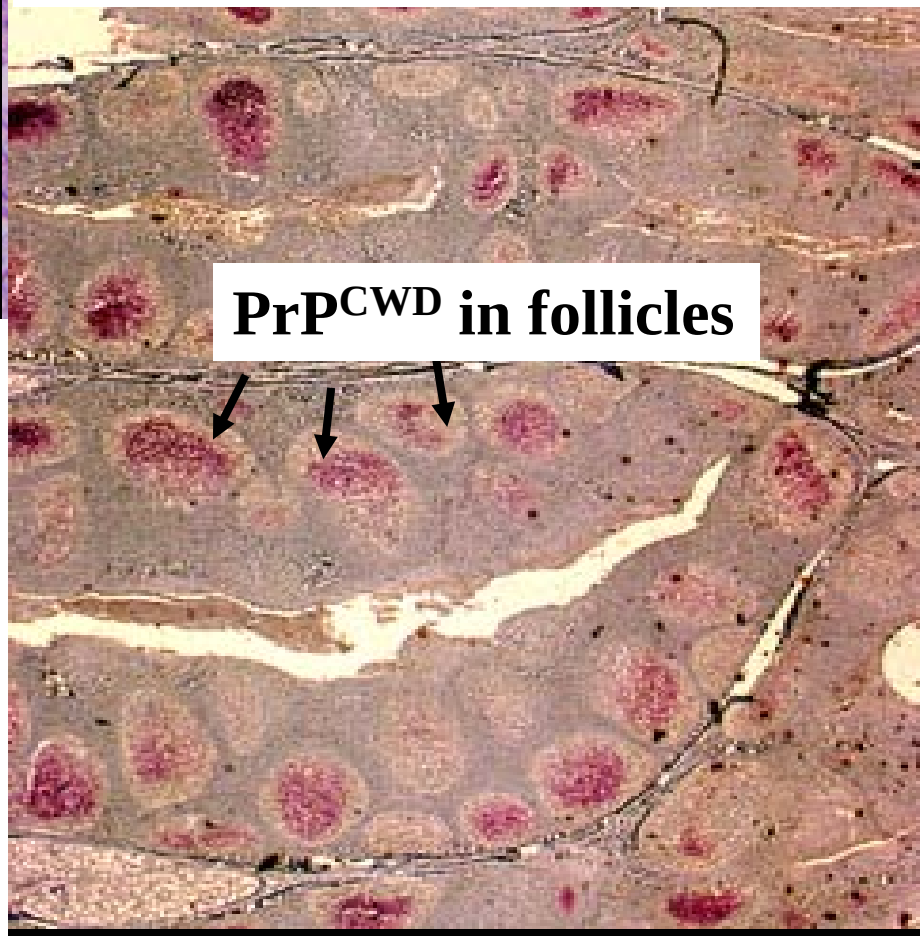
Courtesy of Dr. Michael Miller, CO Div. Parks & Wildlife

CWD detection:

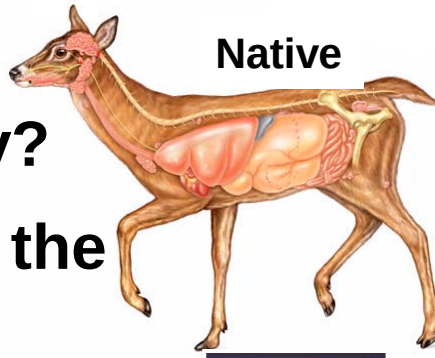
Medulla, obex



Ante-mortem detection based on lymphoid tropism--tonsil or rectal tissue



1. CWD transmission and pathogenesis:

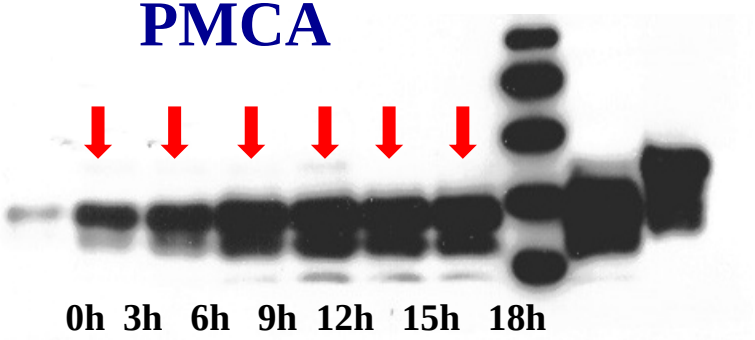


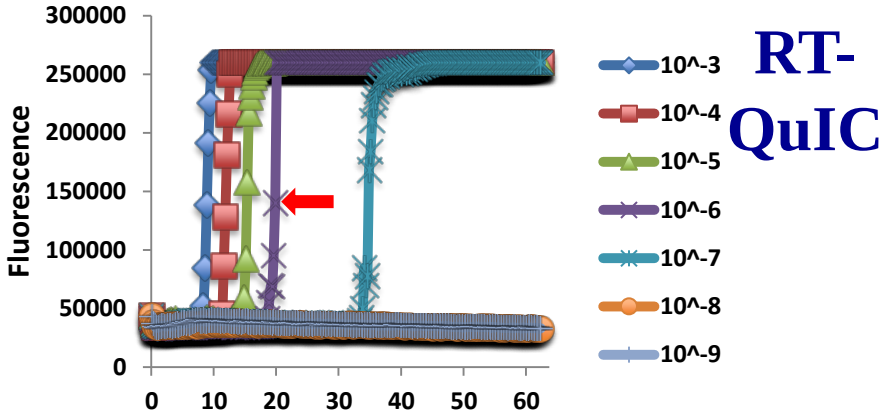
Tg(cerPrP)

- Why/how is CWD transmitted so efficiently?
- How do CWD prions enter, traffic, and exit the body across mucous membranes?
- Prion aerosol infection?
- Repeated low dose exposure?
- Do tissue-specific prion variants exist?

2. Species/transmission barrier:

- Are sympatric predator and contact non-cervid species susceptible to CWD prions?
- Does trans-species infection alter the species barrier?
- Can this be predicted in vitro?



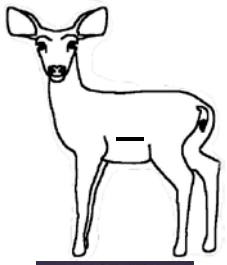


3. In vitro detection/conversion assays:

- Protease-sensitive infectious prions
- More sensitive detection/diagnosis?
- Prion cross seeding--human AD/PD/ALS?
- Screen drugs and inactivators
- Process of conversion/amplification

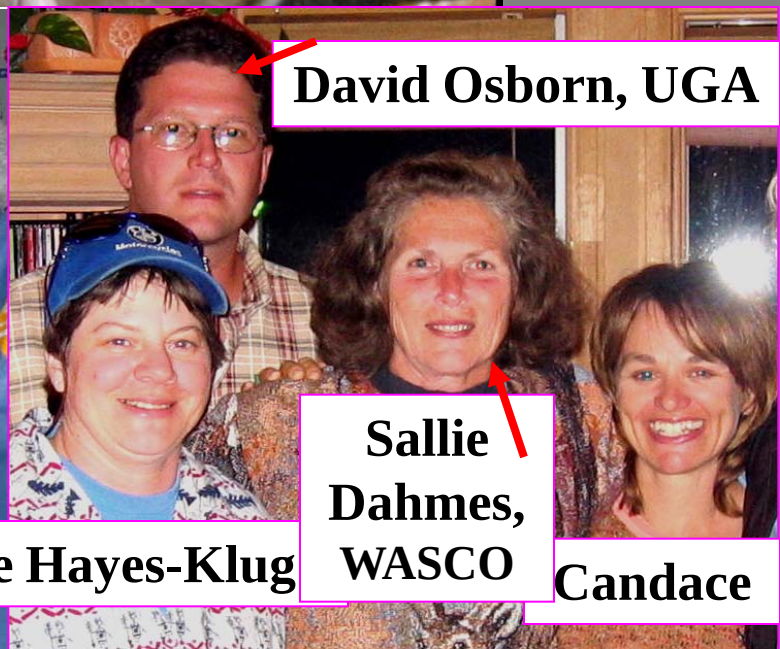
4. Prion vaccination:

- PrP^{c} immunization with a *Salmonella* vector



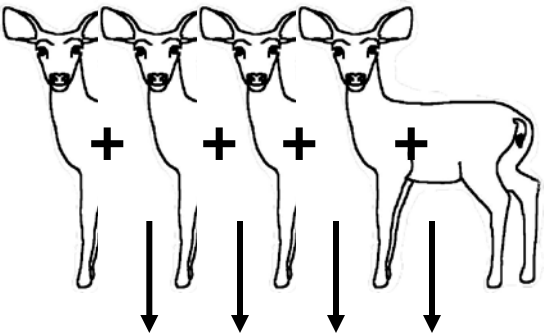
- **Point of exposure longitudinal studies, serial collections**
- **CWD-free, hand-raised, human- and indoor-adapted deer**
- **Whitetailed deer fawns sourced from Univ of Georgia**

We work with CWD *as if* has potential to infect humans



CWD prion transmission and shedding: general study design

CWD+ donors



Tonsil and rectal tissue biopsies to detect infection

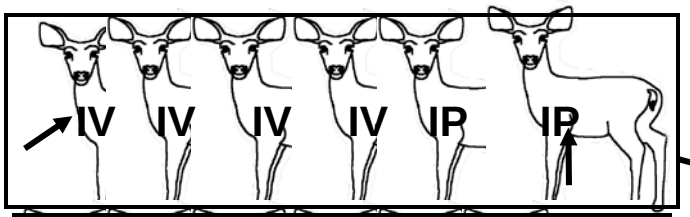
Blood

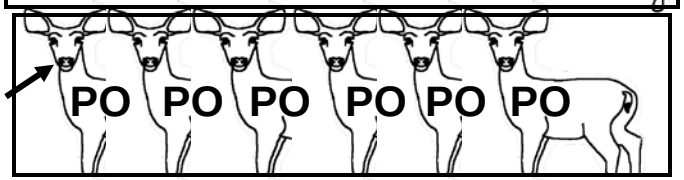
Saliva

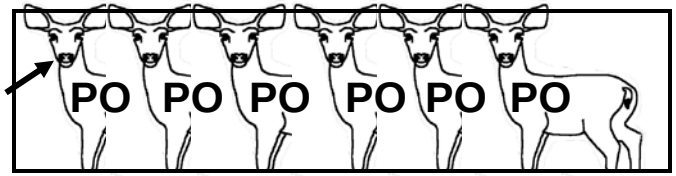
Urine/feces

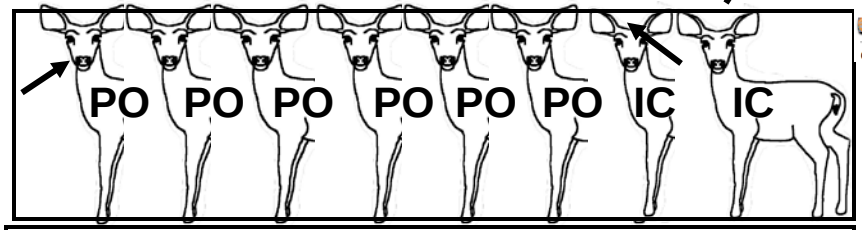
Brain/other

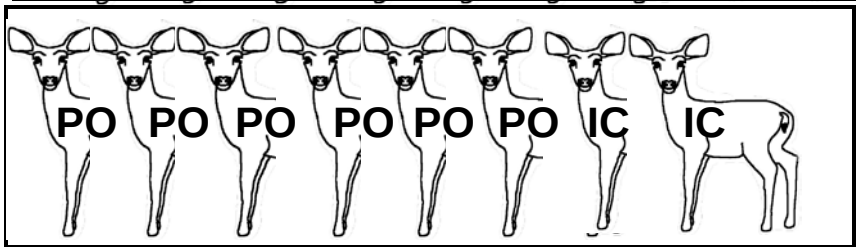
Neg controls



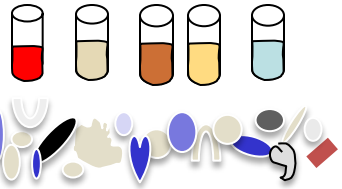




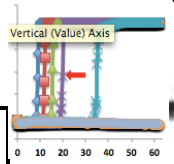




Tissues, body fluids & excreta

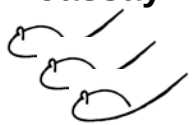


QuIC



PMCA

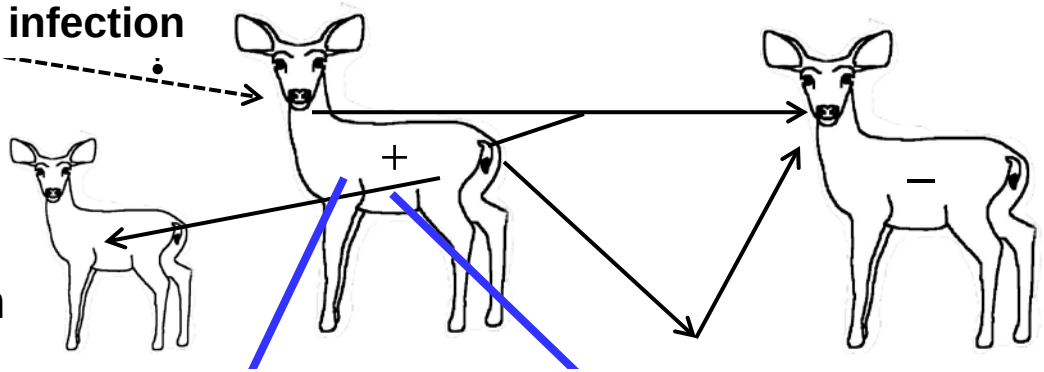
Bioassay



How are CWD prions shed and transmitted in deer?

?

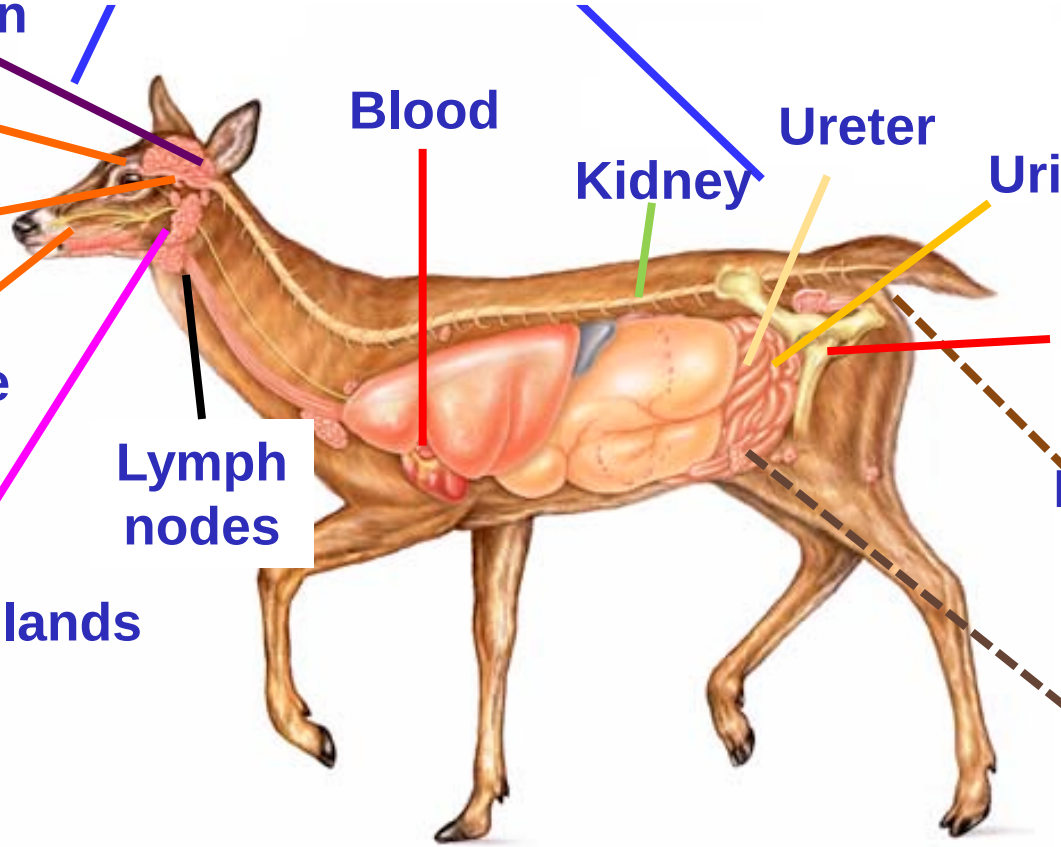
Oral infection



Mother-to-offspring transmission

Direct or indirect horizontal transmission

Prion peripheralization and excretion



Brain

Conjunctiva

Nasal mucosa

Oral/tongue

Salivary glands

Lymph nodes

Blood

Kidney

Ureter

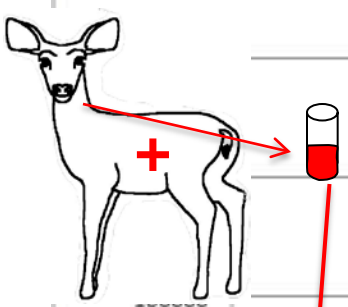
Urinary bladder

Bone marrow

Rectum

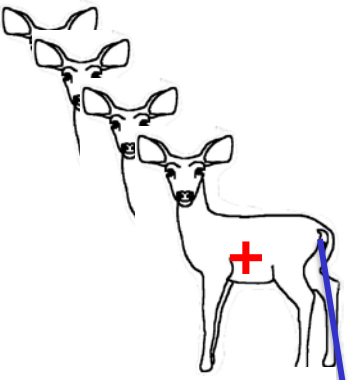
Intestine

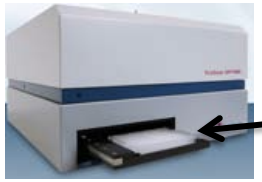
RT-QuIC detection of CWD prions in deer blood



rPrP substrate

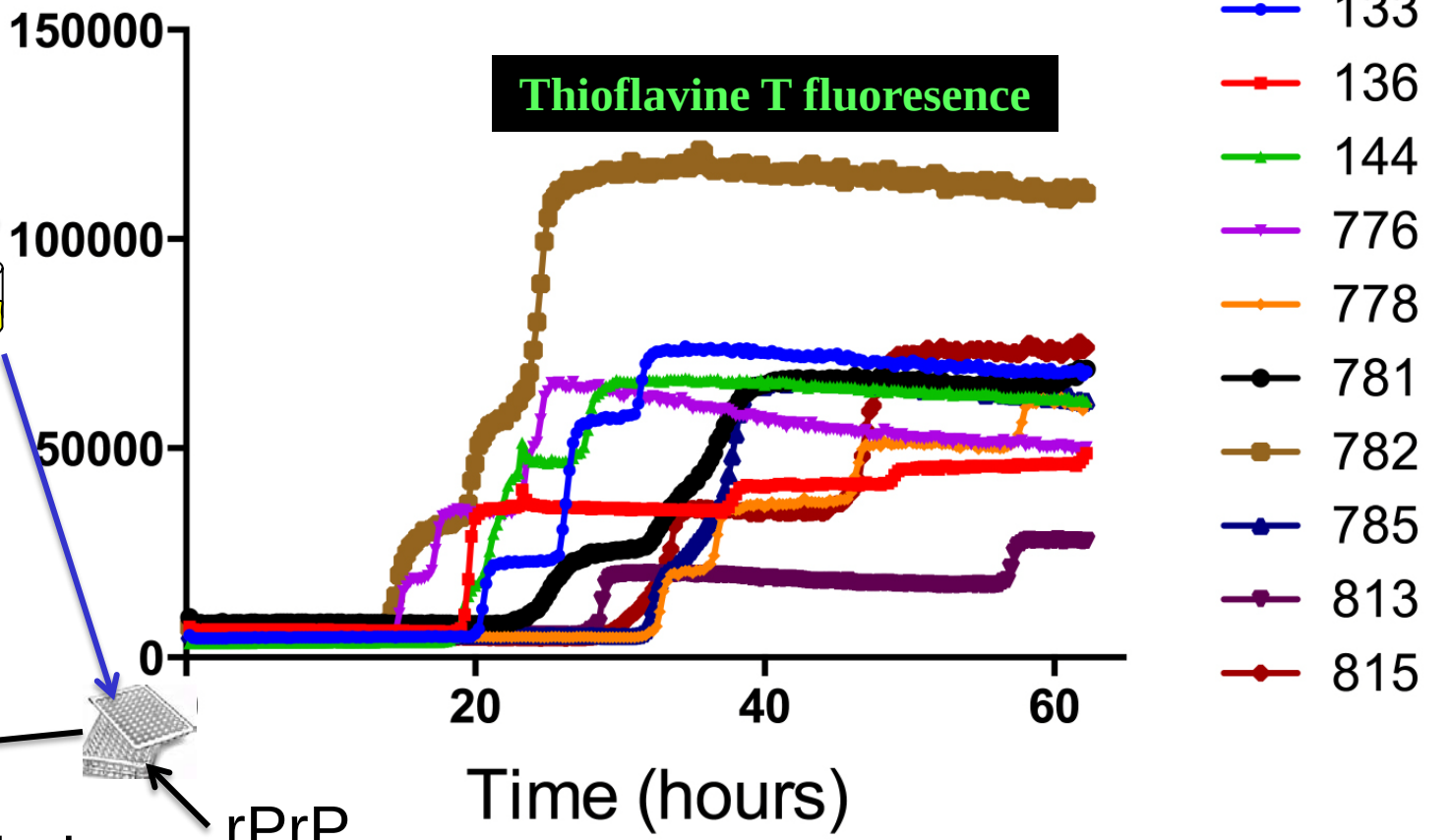
RT-QuIC detection of prions in deer saliva



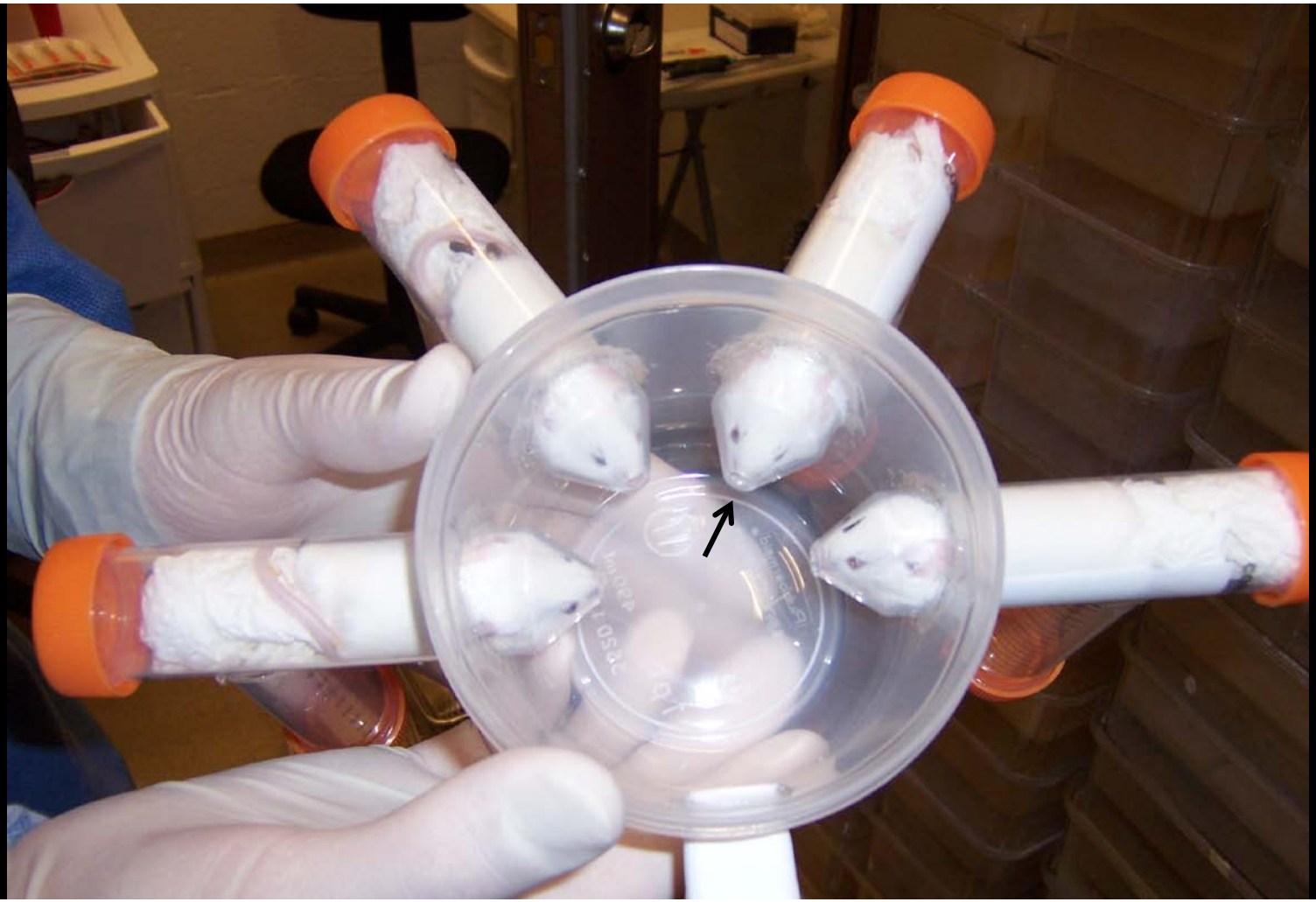


Fluorimeter/shaker

rPrP substrate



Are prions transmissible by aerosol? Yes



Chamber for nose-only exposure of cervid PrP transgenic mice

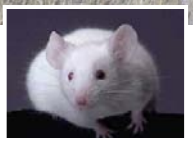
Some findings with CWD (and scrapie) prions:

1. **Infectious prions are in saliva, blood, urine, muscle, feces**
   - **Detected by bioassay and new in vitro methods**
   - **Most tissues contain prions**
2. **Environmental/fomite transmission without animal to animal contact proven**
3. **CWD prions are shed from mucosal excretory/secretory tissues. Perhaps adapted for efficient mucosal infection?**
4. **CWD are transmissible by aerosol and through minor oral lesions**

1. CWD efficient transmission:

- Effective prion peripheralization
- Transfer to body fluids and excreta
- Trans-mucosal shedding
- Mucosal crossing to establish infection

Native



Tg(cerPrP)

2. CWD species barrier:

- **Sympatric and/or predator non-cervid species susceptible to CWD?**
- **Alteration of the CWD host range?**
- **Adaptation -> peripheralization -> shedding/horizontal transmission?**

Loveland CO (leaving home in AM….)

Summary re. CWD prion species barrier:

1. **Some sympatric non-cervid species are susceptible to CWD (by exp. inoculation)**
   - **Voles, *Peromyscus spp* mice, ferrets, hamsters, cats**
2. **Trans-species amplification of CWD prions (in vivo or in vitro) generates infectious prions with altered host range**
3. **Trans-species adaptation is associated with oral susceptibility and alteration of TSE phenotype**

Summary re. prion biosafety:

1. Prions are misfolded cellular proteins that can also become infectious agents
2. Prions adhere to most surfaces and are difficult, but not impossible, to inactivate completely
3. Species crossing by prions is rare, but also remains unpredictable
4. Trans-species adaptation may be associated with alteration of species barrier
5. Prion-like protein misfolding is associated with the major human neurodegenerative diseases

People responsible for CWD work:

Nicholas Haley

Timothy Kurt

Davis Seelig

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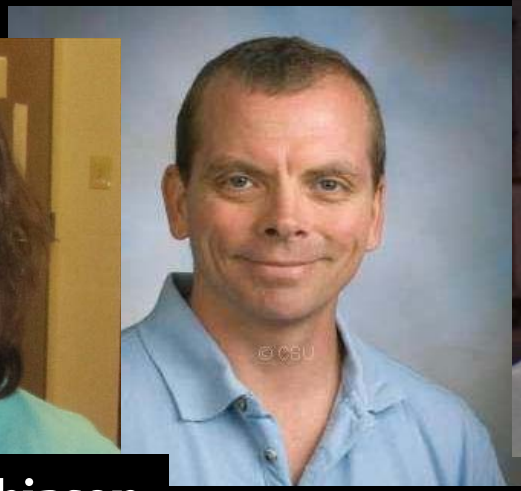
UCSD

Davin Henderson

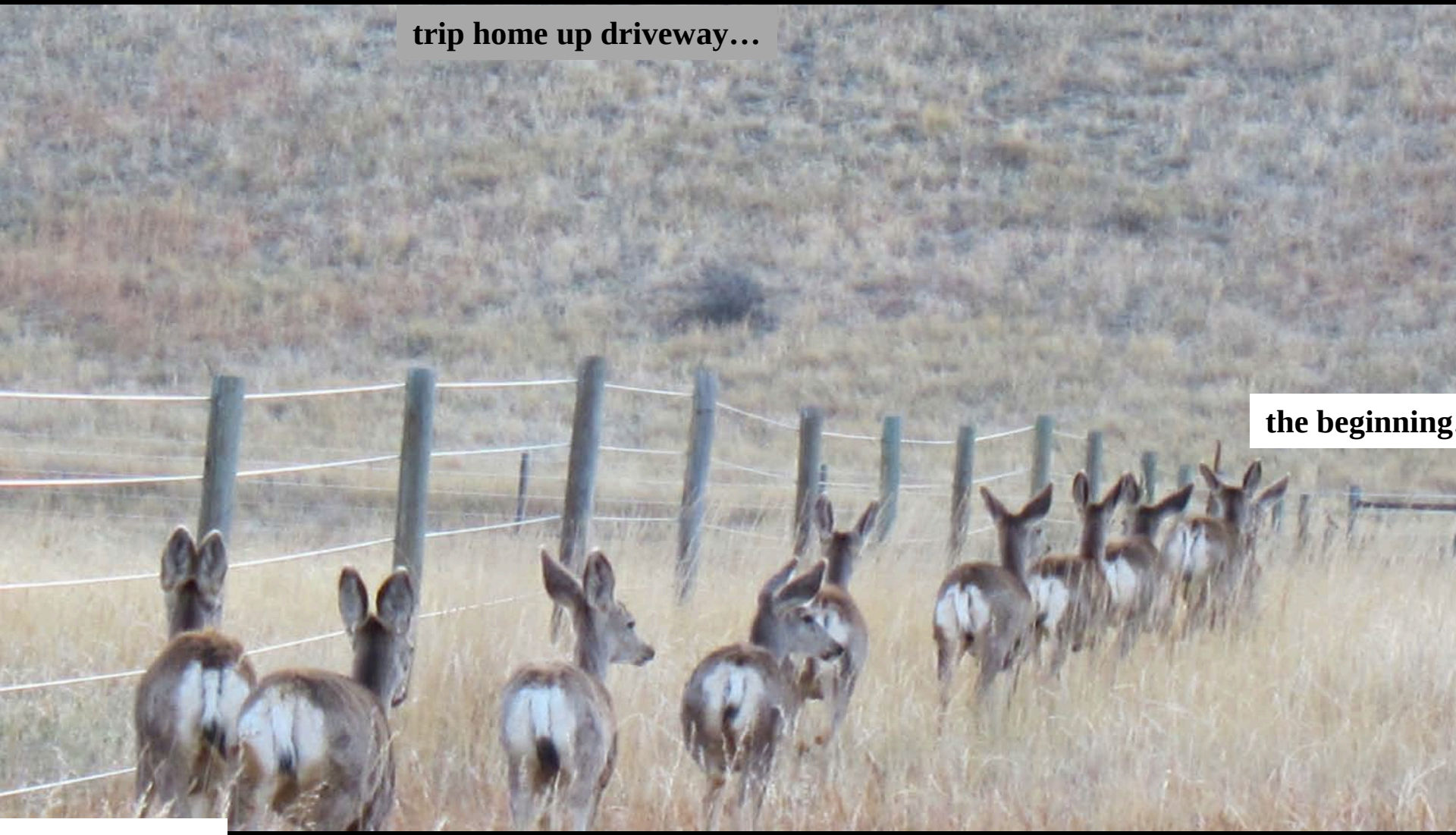
Amy Nalls

Candace Mathiason



Mark Zabel

Glenn Telling



the end…