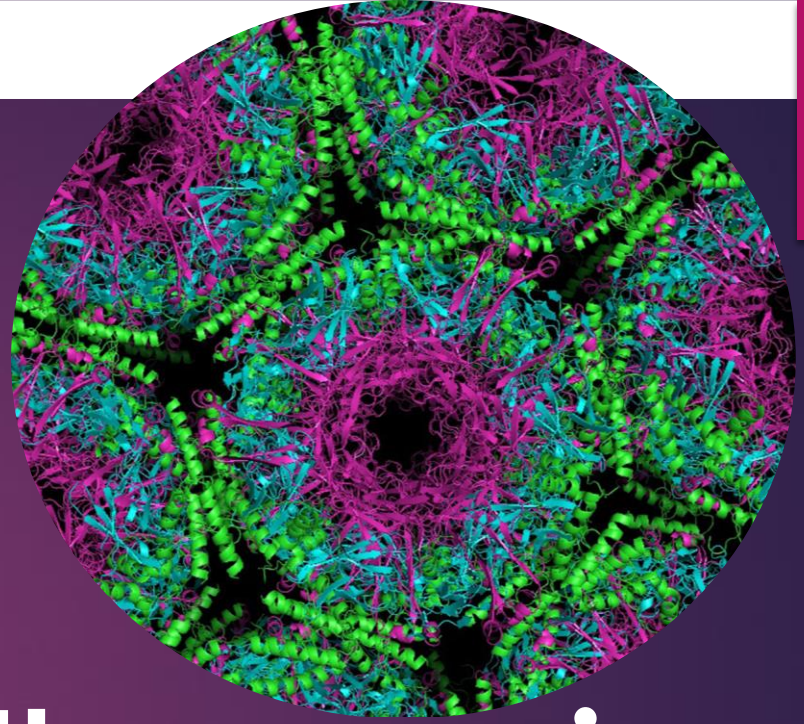


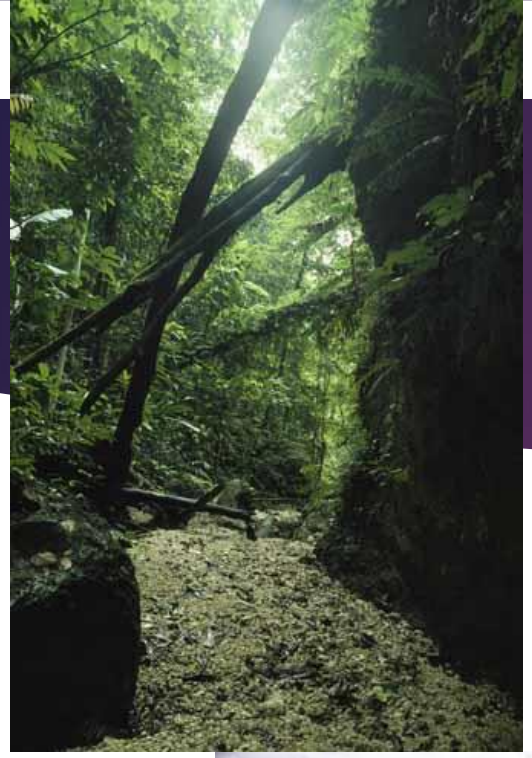
Prion Unveiled: Understanding Pathogenesis and Ensuring Biosafety

ALANA STERKEL, PHD, D(ABMM), SM(ASCP)^{CM}
ASSOCIATE DIRECTOR, CDD
WISCONSIN STATE LABORATORY OF HYGIENE
ASSISTANT PROFESSOR, UNIVERSITY OF WISCONSIN- MADISON

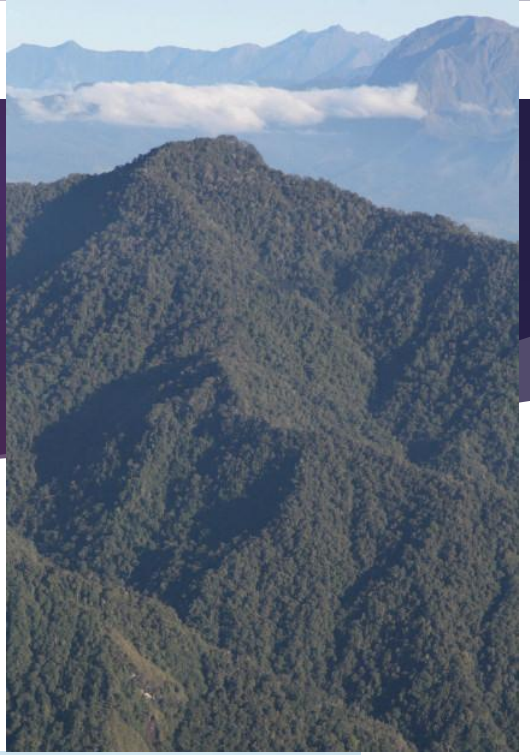
Outline

- The sensational discovery of human prion disease
- Modern disease
- Diagnosis
- Laboratory safety

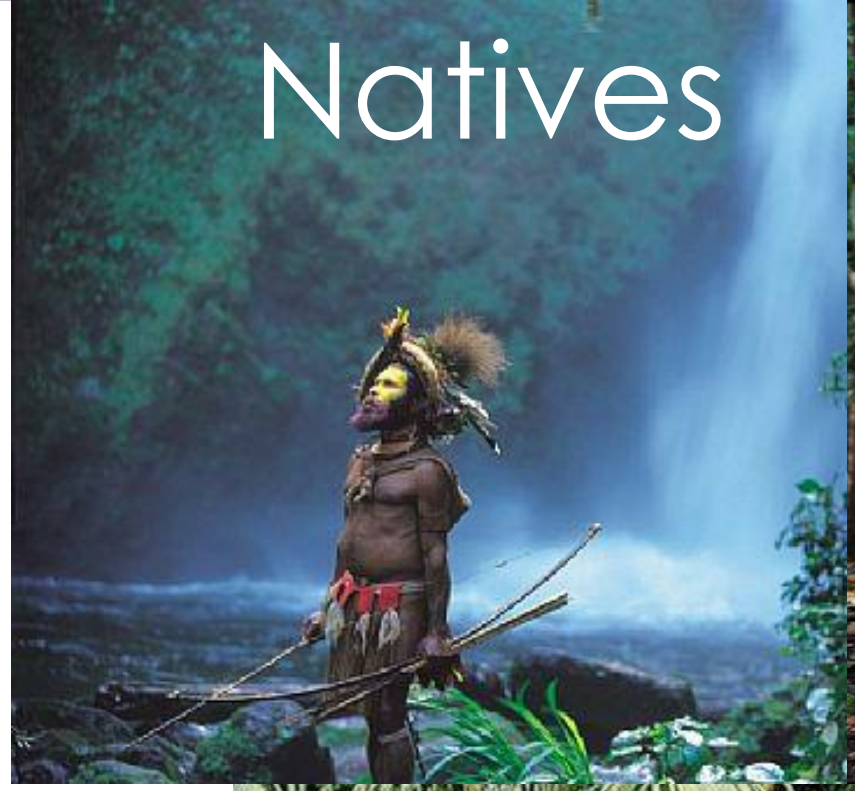
Papua New Guinea

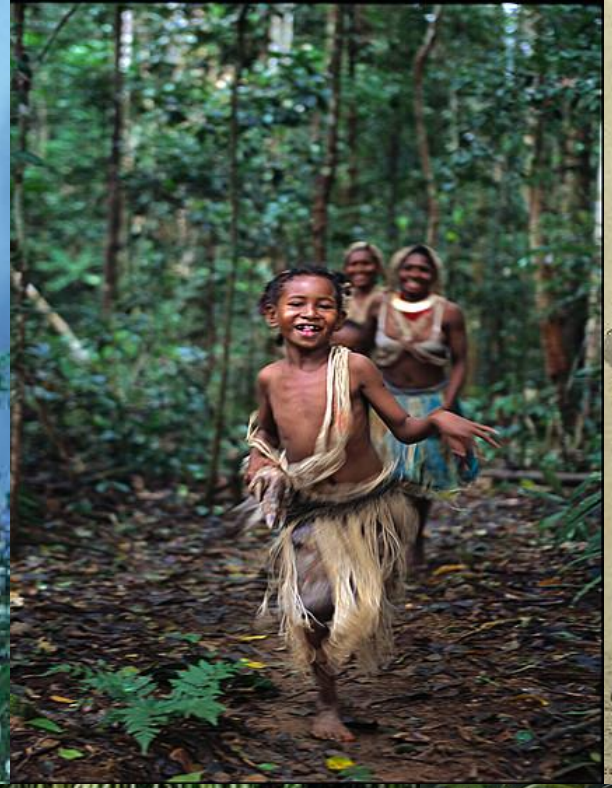


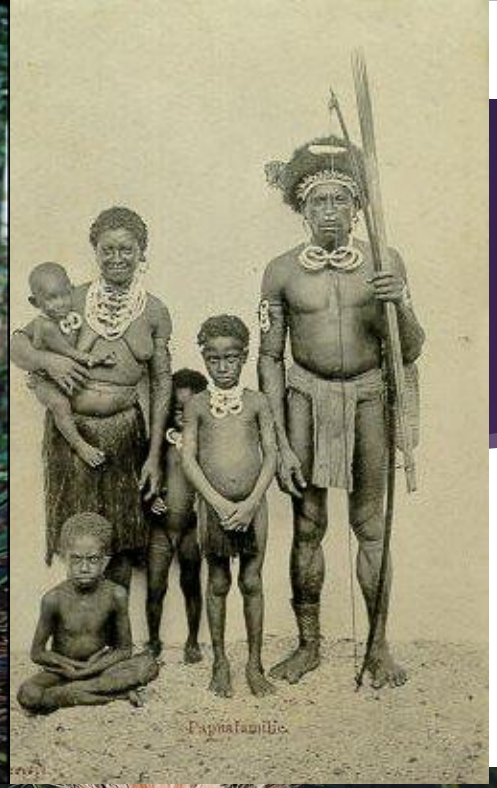
- Dense Jungle
- High Mountain ranges
- Small communities
- First exposed to “society” in 1884



Natives



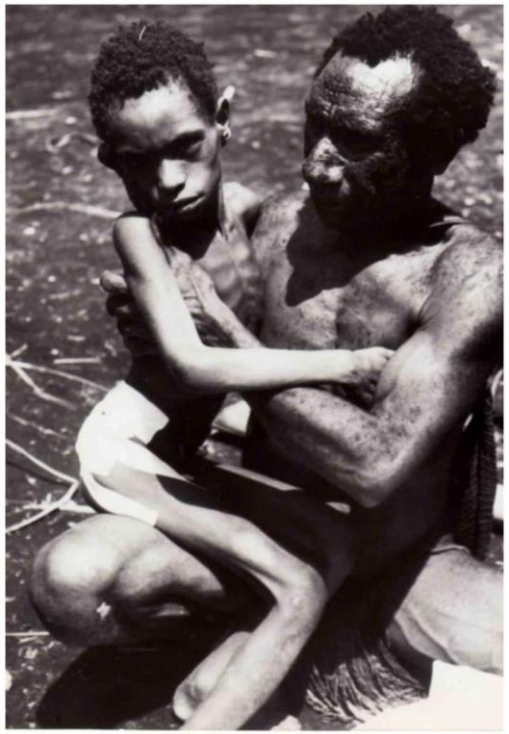




Kuru (early 1900s)

- ~8000 people in South Fore
- 1450 dead over 7 years
- 3x more common in Women
- From first symptoms to death in 4-12 months

Stages of Kuru



- Stage 1: Ambulant
  - Unsteady gait, voice, hands, and eyes
  - Tremors and slurred speech
- Stage 2: Sedentary
  - Can no longer walk
  - Severe tremors and muscle jerks
  - Emotional instability, outbursts of laughter*
- Stage 3: Terminal
  - Tremors and jerks worsen
  - Loss of ability to sit upright
  - Extreme slurring or speech
  - Incontinence
  - Severe ulceration (due to immobility)
  - Difficulty swallowing

*Coined "the laughing disease" by the media

Resulted in starvation or choking death

Gadjusek (1973)

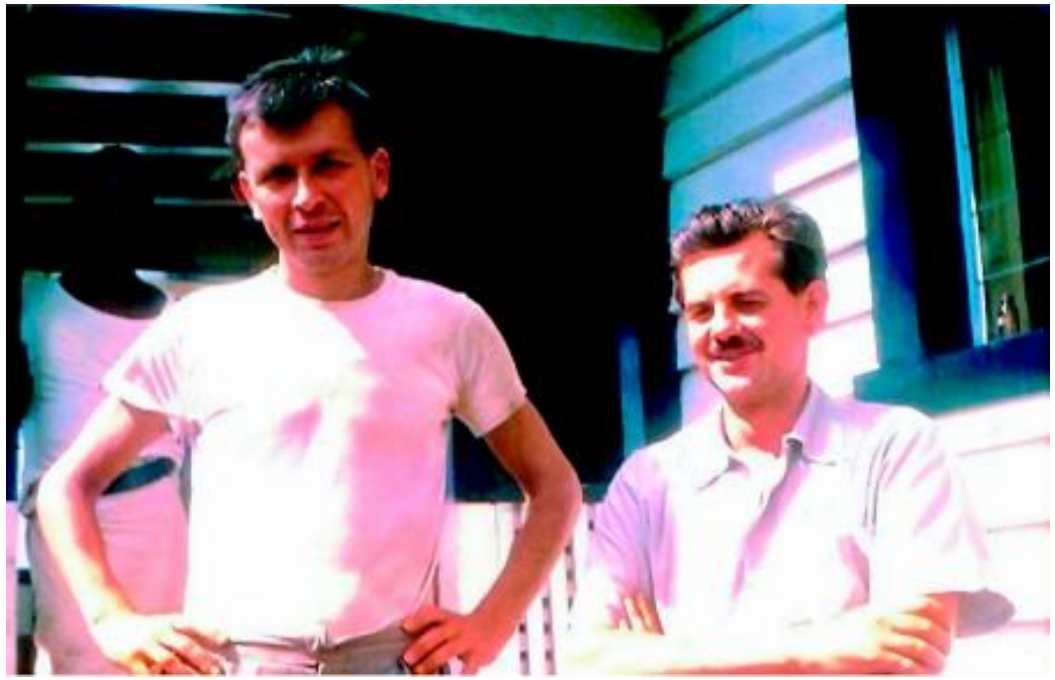
Outbreak Hysteria

"*I was still very young when I saw [kuru] and even after we treated it there was no help. Everyone was falling apart. [Kuru victims] were aware there was no cure and that they would die. It wasn't just one person that this sickness came to – there were about three in a house line and then after they died there would be another three. It was…ongoing…there were many deaths. Once a [person]…was affected by kuru [their] family would think that the clan had poisoned [them] and they would start…shooting at each other and that made it worse. It was chaos ! (Taurubi)* .

Big Names in Kuru

- Dr. Vincent Zigas
- Carleton Gajdusek
- Shirley Lindenbaum

- Genetic?
- Infectious?
- Poison?



Carleton Gajdusek and Jack Baker outside Jack's house in Okapa in 1957

Lucy M. Hamilton Reid

Investigating Foods

- Conducted surveys
- Watched the preparation and eating of meals
- Collected specimens of foods, condiments, and medicine
- Investigated anything that came into contact with food

- Men would hunt for meat
- Young boys ate insects and small animals caught in the forest
- Women farmed and ate more vegetables

Mumus

- Underground cooking pits
- Bamboo for cooking tubes
- Bark and leaves for plates

Funereal Cannibalism

- Documented by Anthropologists years earlier.
- Didn't want to leave their dead cold and alone in the ground
- Way of using all protein sources
- Special right of women

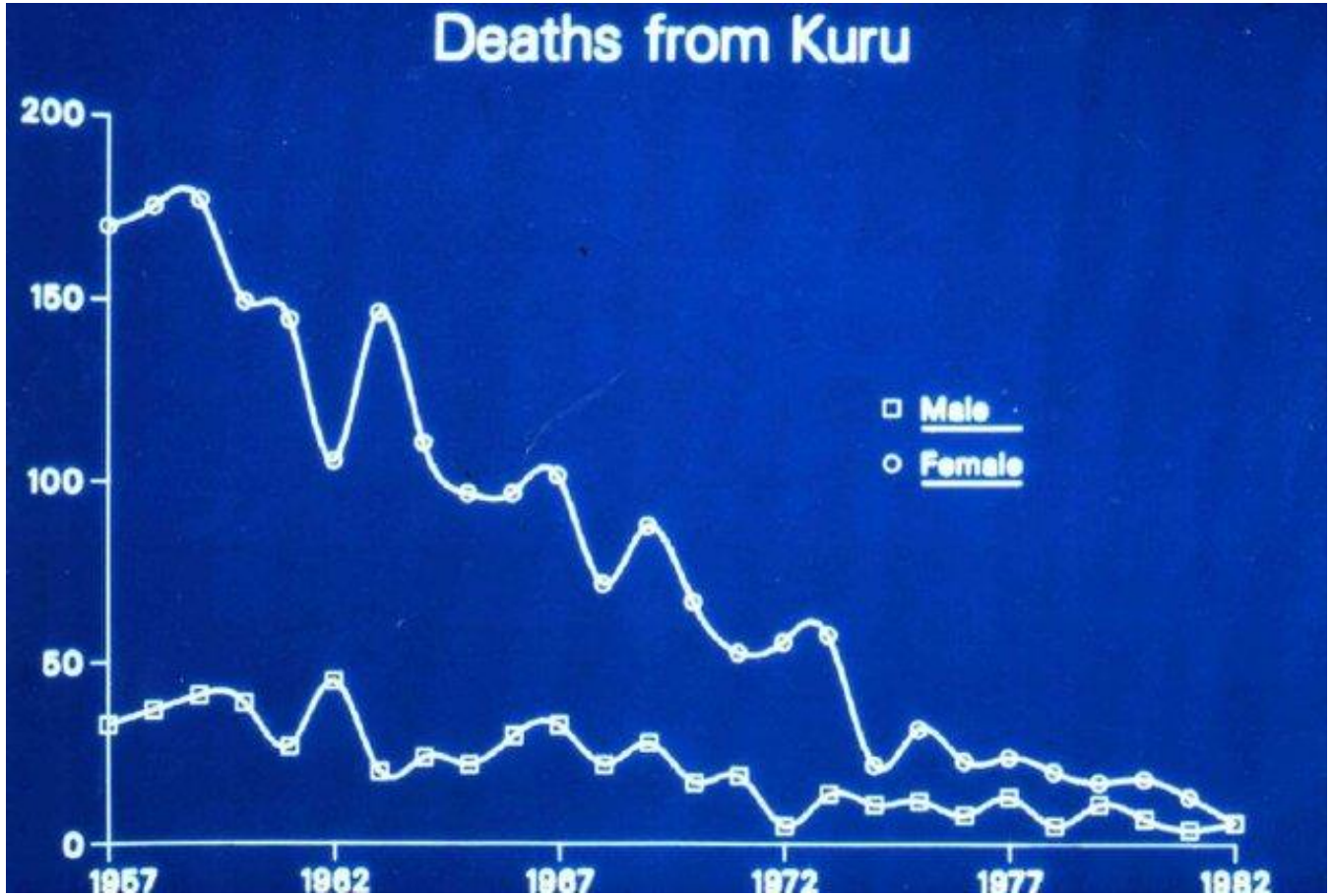
Testing the Hypothesis

- Brain matter from Kuru victims was fed to chimpanzees
- A similar disease was finally found many months later
- Identifying the source of the disease

First Chimpanzee affected with Kuru

https://www.researchgate.net/publication/269114598_Kuru_A_Journey_Back_in_Time_from_Papua_New_Guinea_to_the_Neanderthals%27_Extinction/figures?lo=1

Impact of the Discovery

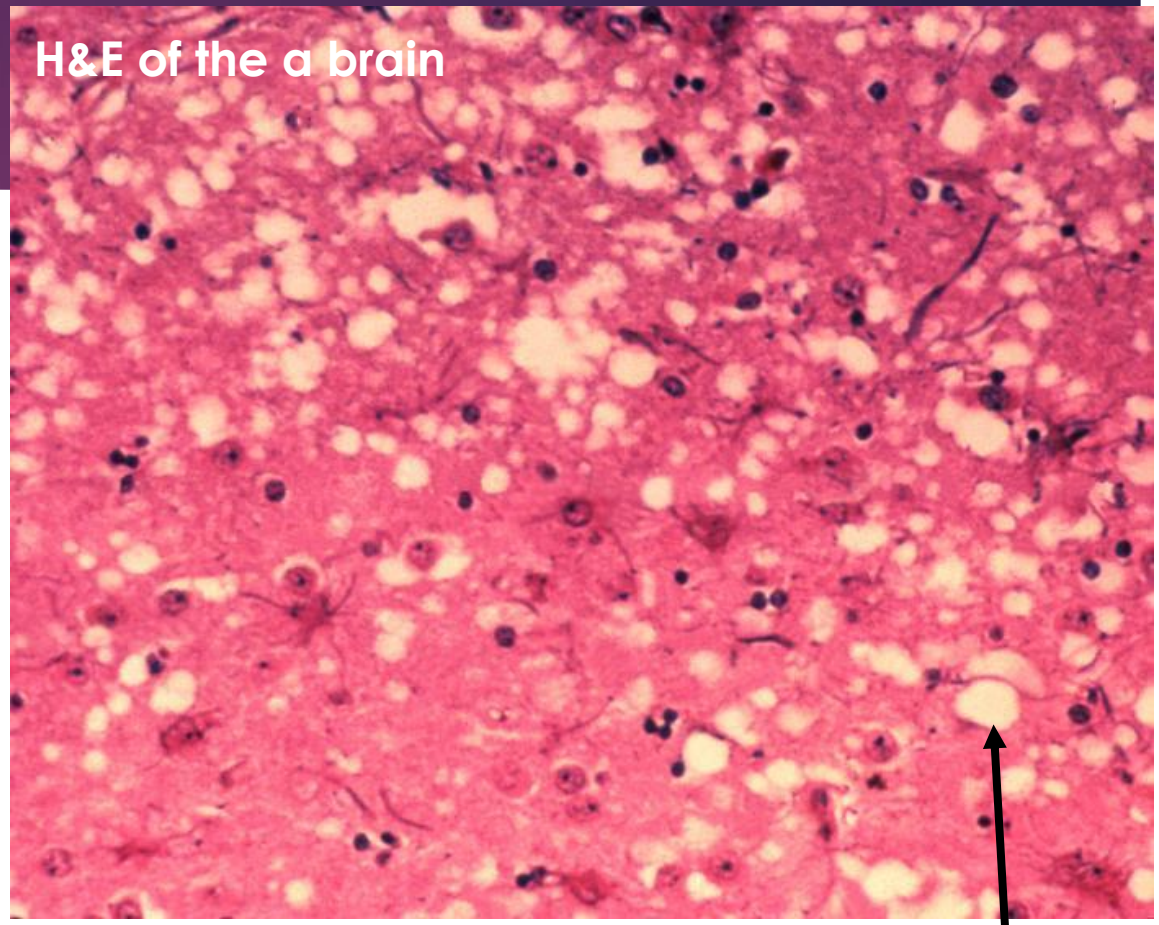


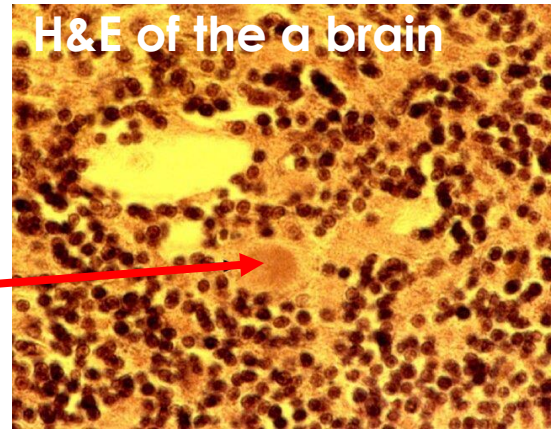
*Now considered extinct

Understanding the Disease

Transmissible Spongiform Encephalitis (TSE)

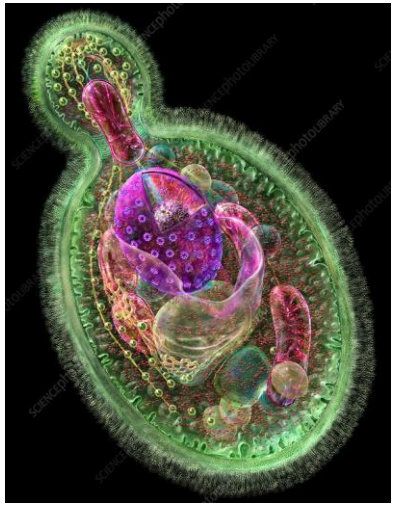
- Sponge-like holes in the brain
- 2-23 year incubation in humans
- Viral-like plaques in the brain
- Termed a "Slow Virus"
- Gajdusek won the Nobel Prize in Medicine-1976



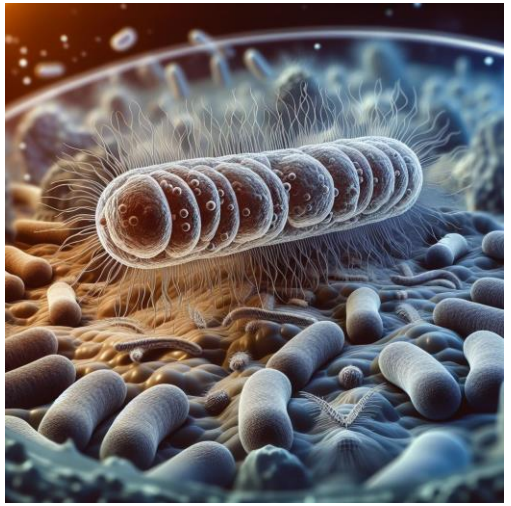


Types of Pathogens

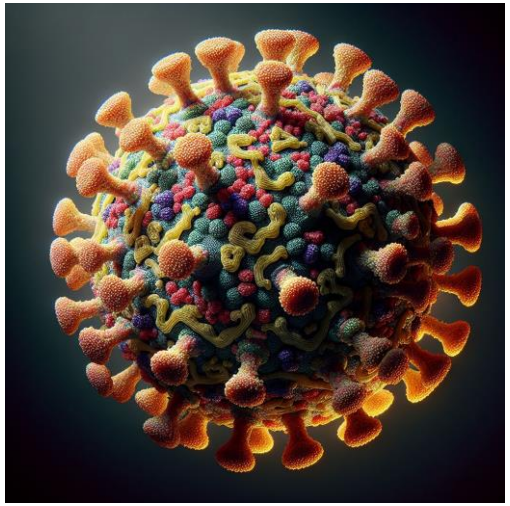
Parasites



Fungi



Bacteria



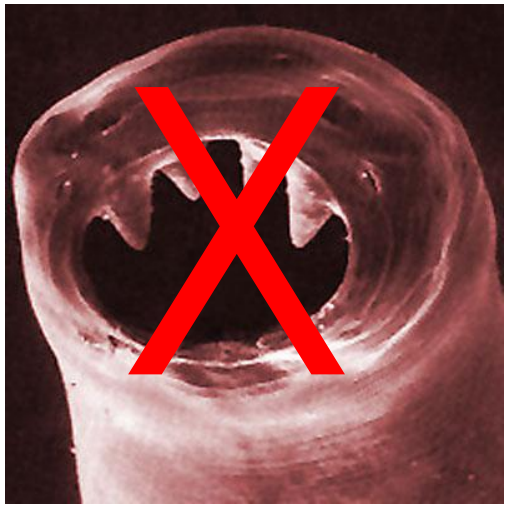
Viruses

Is it Infectious or Hereditary?

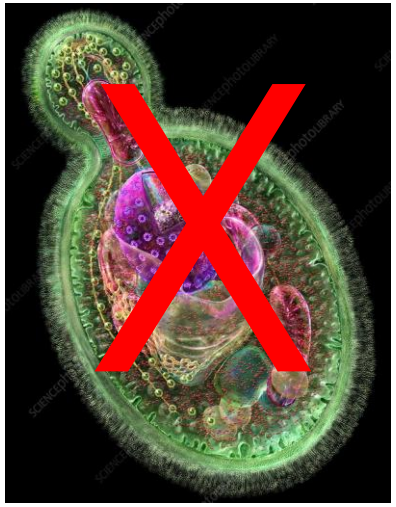
- Extremely resistant to radiation and heat
- Does not cause an inflammatory response in the body
- "Biochemical nightmare" to purify
  - Not possible to separate by sucrose gradients or chromatography
- Mouse models of infection took years to develop disease
- Eventually, Prions were discovered
  - Prusiner awarded a Nobel prize in 1982

Prusiner (left) being awarded a Nobel prize,1982

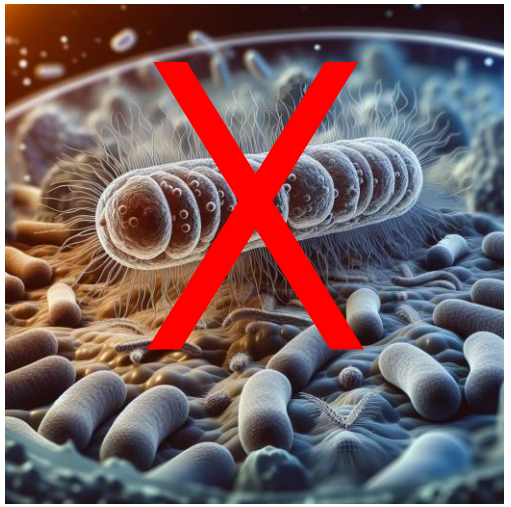
Types of Pathogens



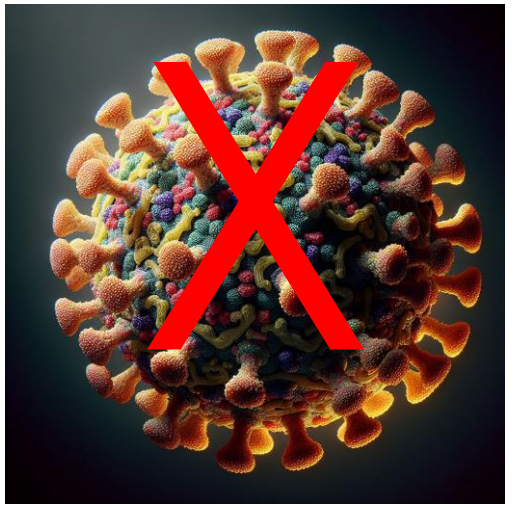
Parasites



Fungi



Bacteria

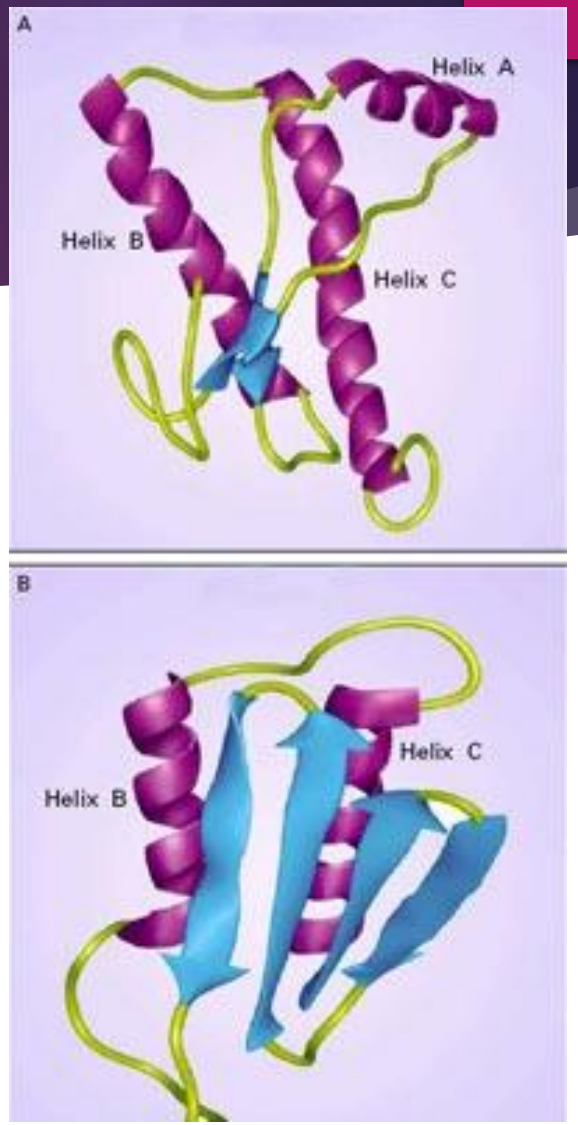


Viruses

*No nucleic acids involved!

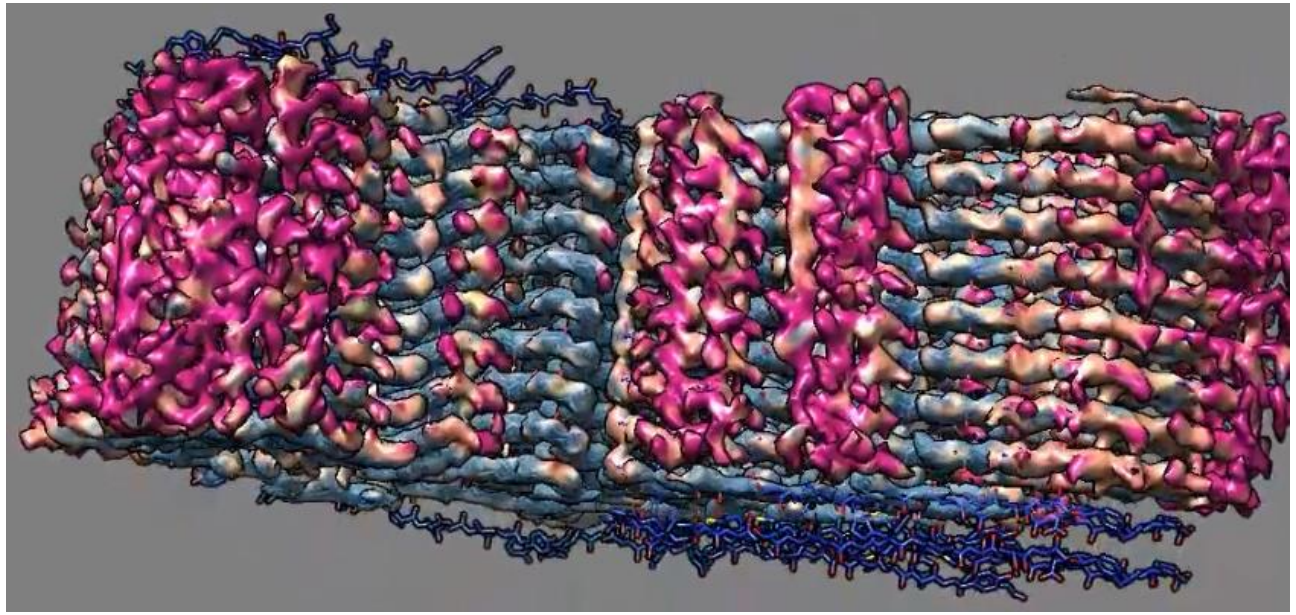
Prions
proteinaceous infectious particles

- Naturally occurring protein
  - **Pr**ion **p**rotein, **C**ellular = **PrP^{c}**
- Mutant form
  - **Pr**ion **p**rotein, **Sc**rapie isoform = **PrP^{Sc}**

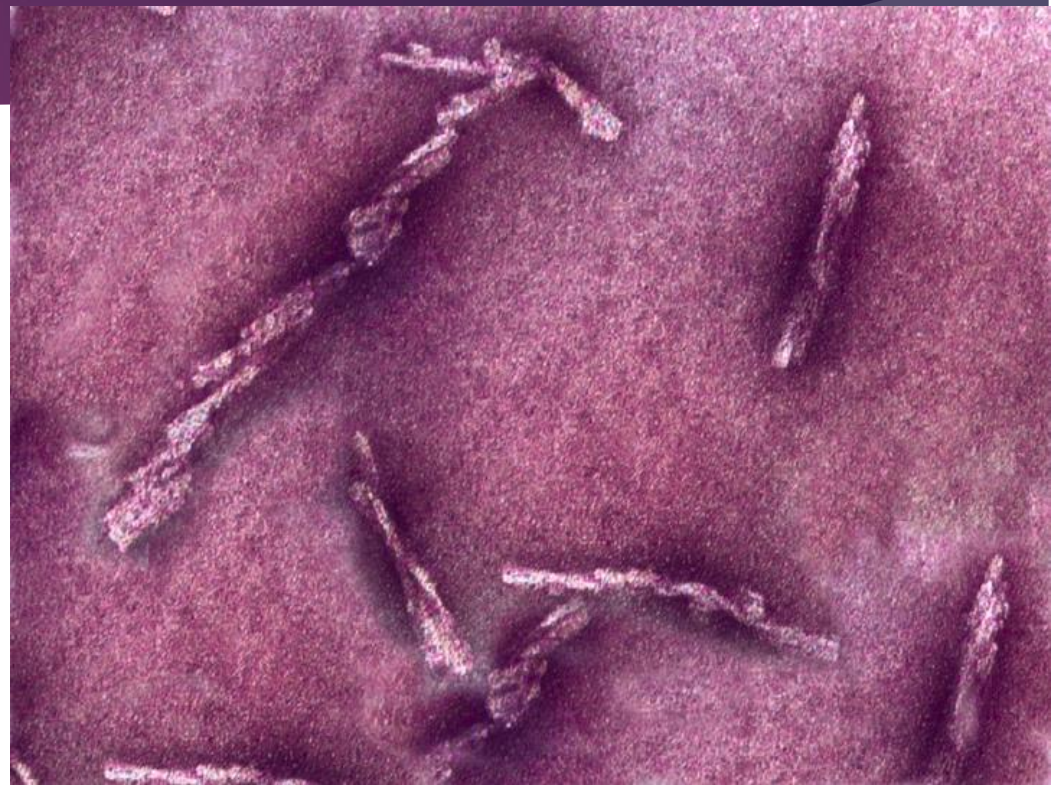


https://thedaily.case.edu/first-atomic-level-imaging-of-lethal-prions-provide-sharpened-focus-for-potential-treatments/

Cascading mis-folding of neighboring proteins



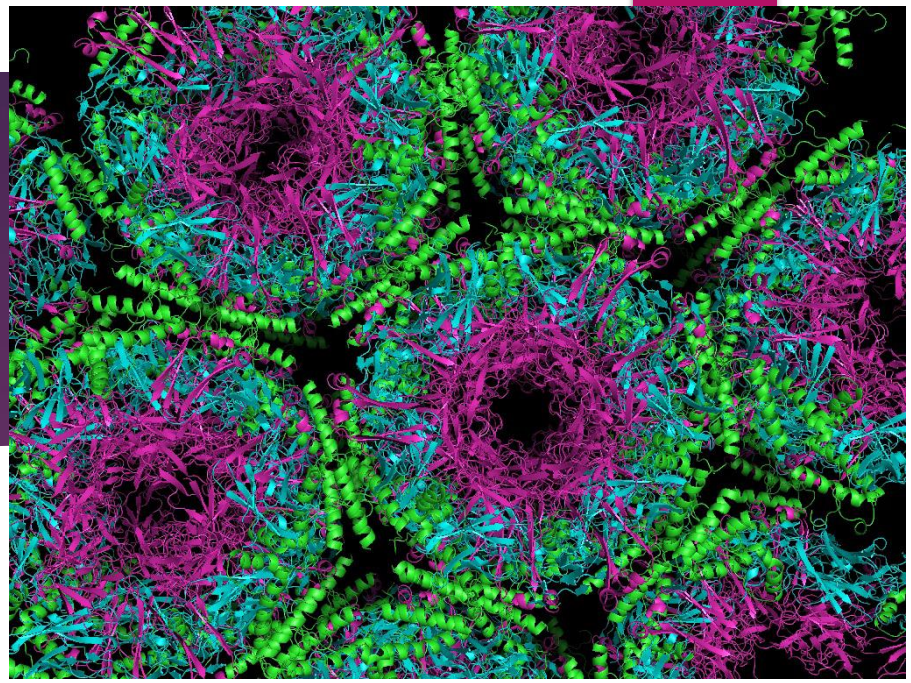
Prions stack

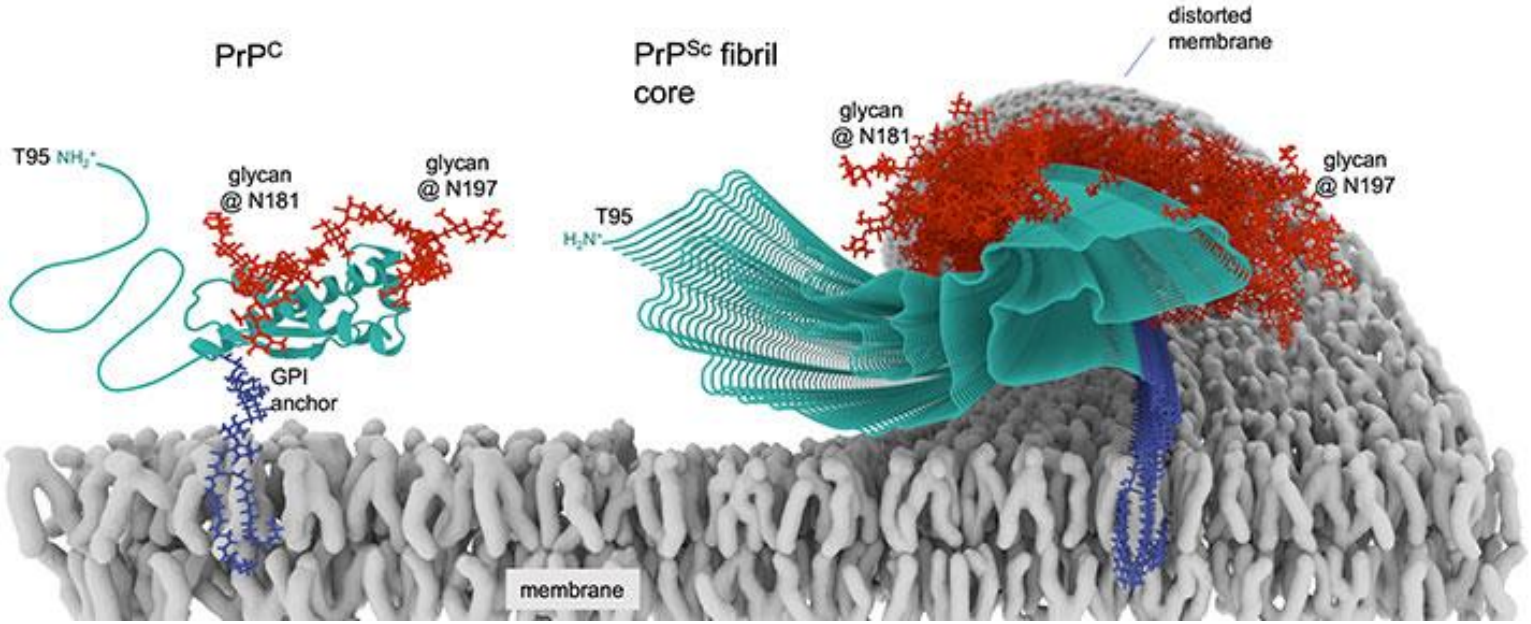


Prion Fibrils

Normal Prion Proteins

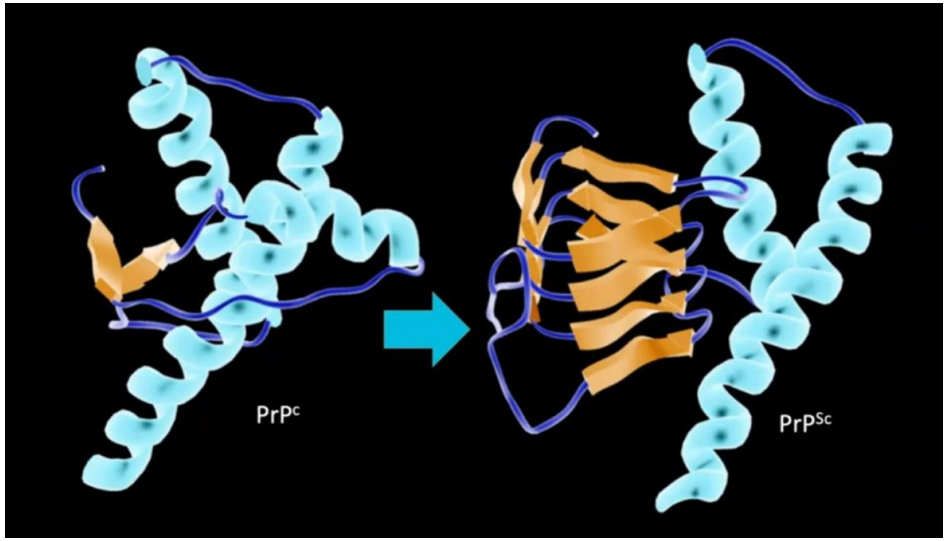
- Located mainly on cellular membranes of neurons
- Involved with cell adhesion and structure





What we know so far

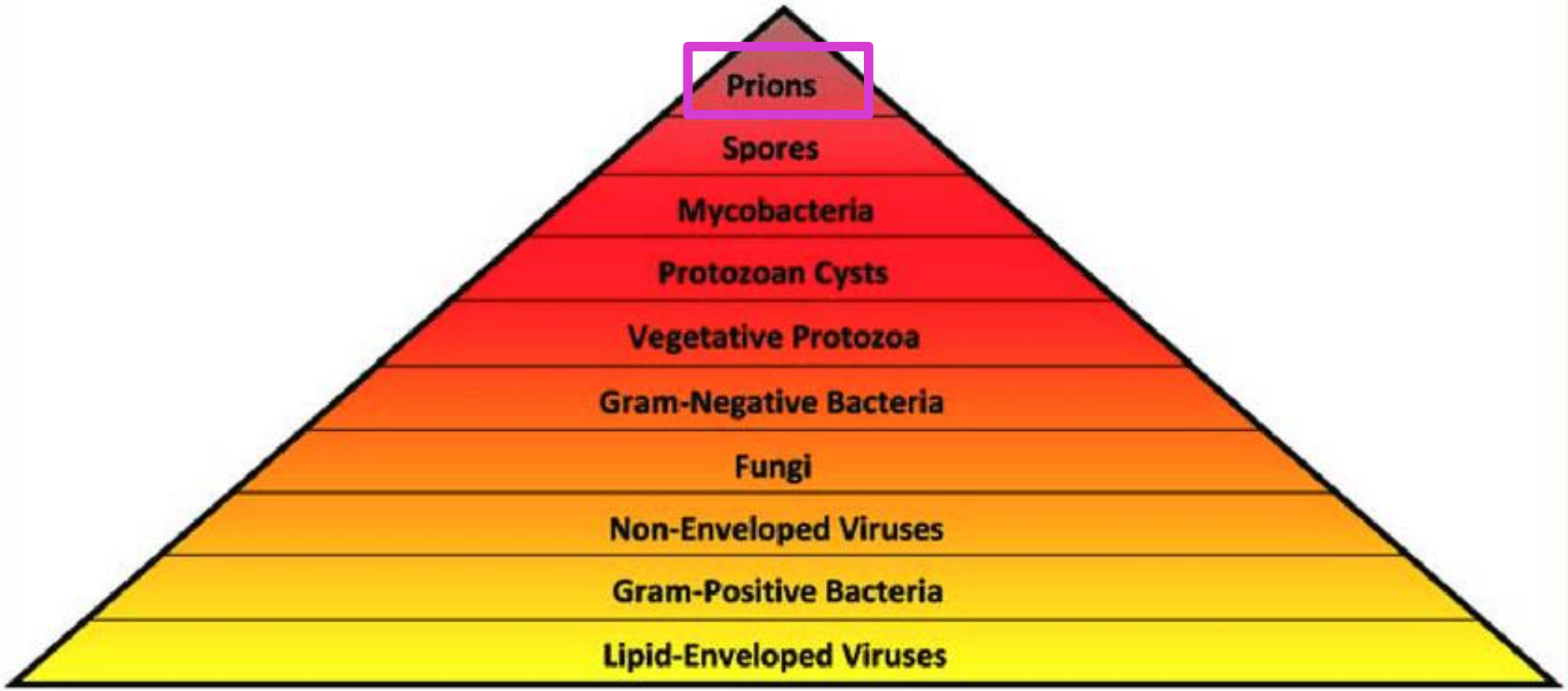
- Devastating neurodegerative disease
- Uniformly deadly
- Infectious but does not cause inflammation
- Caused by a whole new class of "pathogen"
  - Prorogates but does not replicate
  - Would not exist without a host
  - Is not "alive", has no nucleic acid



Prion Characteristics

- Incredibly stable in the mis-folded form
  - Heat resistant
  - Chemical resistant (alcohol, ammonia, bleach, formalin)
  - Radiation resistant
  - Can persist in the environment for years
- Can be induced chemically without infectious particle!

Most resistant to decontamination/sterilisation



Least resistant to decontamination/sterilisation

Transmissible to a wide range of animals

Species	Incubation period (months)
Goat (Capra hircus)	(104)+
Guinea pig (Cavia porcellus)	(27)
Opossum (Didelphis marsupialis)	(22+)
Domestic cat (Felis domesticus)	(59)
Gerbil (meriones unguiculatus)	(24)+
Hamster (Mesocricetus auratus)	(28)
Mous (Mus musculus)	22.5
Ferret (Mustela putorius)	18 – 70.5
Mink (Mustela vision)	45
Sheep (Ovis aries)	(63)+

Number in parenthesis – numer of months elapsed since the inoculation, during which the animals remained asymptomatic.

https://www.researchgate.net/publication/269114598_Kuru_A_Journey_Back_in_Time_from_Papua_New_Guinea_to_the_Neanderthals%27_Extinction/figures?lo=1

Other Prion Diseases

Natural

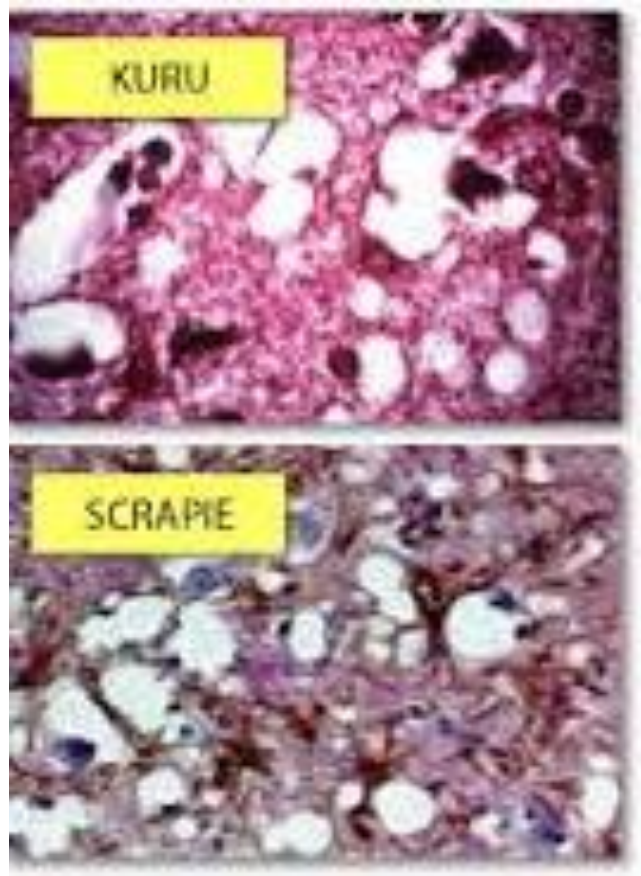
- Scrapie in sheep
- Bovine spongiform encephalopathy in cattle
- Chronic wasting disease in cervids

Iatrogenic (acquired)

- TME in mink (acquired from sheep or cattle)
- FSE in cats (acquired from sheep or cattle)
- And others

Other Prion Diseases

- Scrapie was the first described Prion disease
- Degenerative disease of the nervous system in sheep and goats
- characterized by a progressive loss of coordination and behavioral changes. Causes itching and loss of wool
- May be very ancient
- Despite extensive research and studies, there is no evidence that scrapie prions can infect humans



Bovine Spongiform Encephalopathy (BSE)

- In 1984, mad cow disease, also known as bovine spongiform encephalopathy (BSE), first appeared in cattle in the United Kingdom
- It rapidly evolved into a major epidemic, and by 2004, more than 180,000 bovine cases of BSE had been reported.
- BSE is transmissible to humans through ingestion
- About 233 cases of human disease since it was discovered
- Mad cow disease has since been identified in 23 countries, including Canada and the United States.

Chronic Wasting Disease

- Rocky Mountain elk, moose, and certain species of North American deer, including white-tailed deer and mule deer
- Has been observed in at least 22 states in the United States and three Canadian provinces
- There is no evidence that CWD can be transmitted to humans.

https://journals.asm.org/doi/10.1128/cmr.00046-15

Creutzfeldt-Jakob disease (CJD)

- Brain disorder that leads to dementia
- Very rare. Globally, only 1-2 cases per million/year
- Most often affects older adults
- Always fatal, death usually occurs within a year
- Death usually related to trouble swallowing, falls, heart issues, lung failure, or other infections
- Heritable

- Symptoms:
  - Personality changes
  - Memory loss
  - Impaired thinking
  - Blurry vision or blindness
  - Insomnia
  - Problems with coordination
  - Trouble speaking
  - Trouble swallowing
  - Sudden, jerky movements

https://www.mayoclinic.org/diseases-conditions/creutzfeldt-jakob-disease/symptoms-causes/syc-20371226

Creutzfeldt-Jakob disease (CJD)

- Once symptoms are detected diseases progresses quickly, death usually occurs within 2 years
  - Average survival of 5 months
- There is no effective therapy for prion diseases
  - Antibacterials, antivirals, and antifungals all fail
- Vaccine are ineffective
  - While the three-dimensional conformations of PrPSc and PrPC differ, their amino acid sequences are identical; hence, the pathological accumulation of PrPSc does not induce a classical immune response. Area of research.

Pocchiari et al., 2004

Cases

- In 1974 a patient received a corneal transplant from an cadaver donor
- 18 months later the patient developed CJD and died
- It was later learned that the donor had confirmed CJD

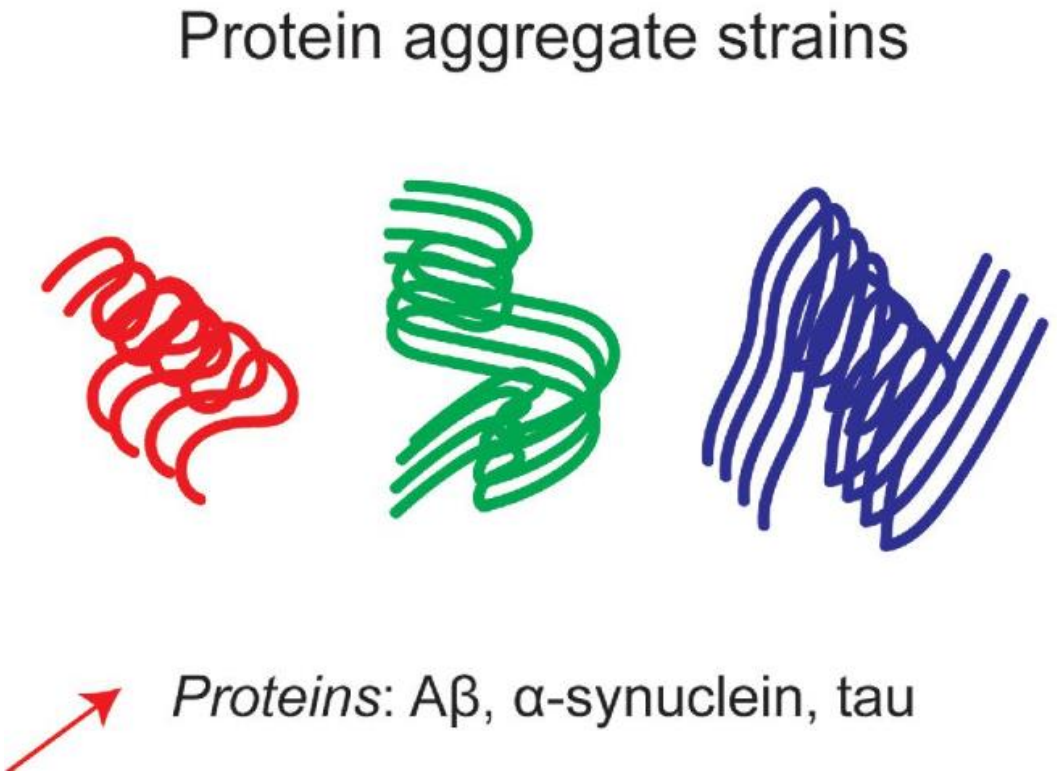
https://europepmc.org/article/med/4591849

- Two cases were reported in 1977.
- CJD developed 2 years after stereotactic EEG recordings.
- The instruments had previously been used in a patient with rapidly progressive dementia and myoclonus, who was later confirmed as having died of CJD.
- The electrodes had been disinfected with ethanol and formaldehyde vapour.
- Hypothesis later supported by transmission of CJD to a chimpanzee 18 months after intradural implantation of the suspect electrodes.

Robert G Will. Acquired prion disease: iatrogenic CJD, variant CJD, kuru. British Medical Bulletin 2003; 66: 255–265

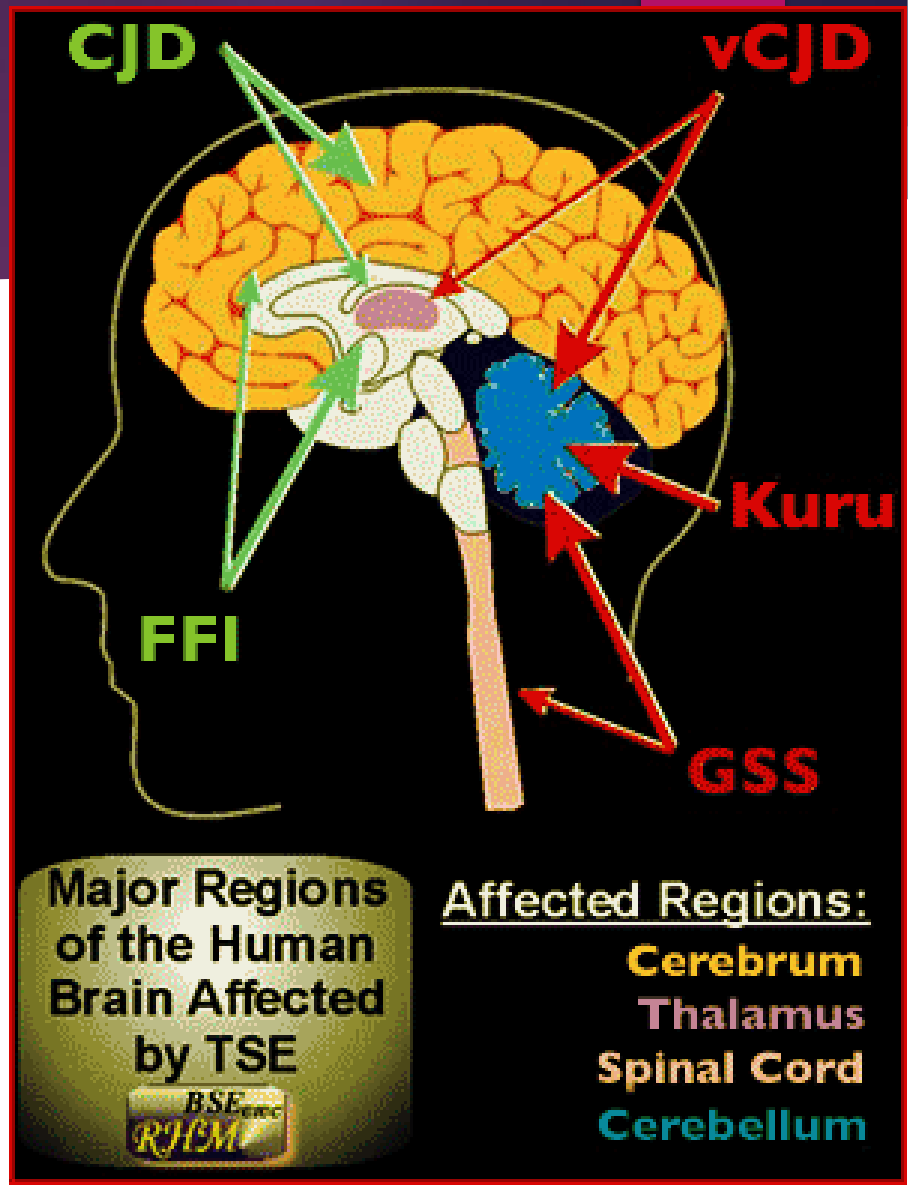
Related Prion Diseases

- All prion proteins
- Small differences in the proteins found in different animals
- Different ways prions can mis-fold, 3 primary morphologies
  - Aβ
  - α-synuclein
  - tau



Human Prion Diseases

- Kuru
  - Acquired: Ingestion (extinct)
- Creutzfeldt–Jakob disease (CJD)
  - Familial: inherited genetic mutation (5-15%)
  - Sporadic: random mutation (~85%)
  - Variant: consuming animals (<1%, ~233 cases)
  - Iatrogenic: surgery, transfusion (<1%, 500+ cases)
- Fatal Familial Insomnia (FFI)
  - Genetic (1 per million annually)
- Gerstmann–Sträussler–Scheinker syndrome (GSS)
  - Genetic (1 per 100 million annually)
- Variably Protease-Sensitive Prionopath (VPSPr)
  - Sporadic (1 per 100 million annually)



bioweb.uwlax.edu/.../strama_lesl/codons.htm

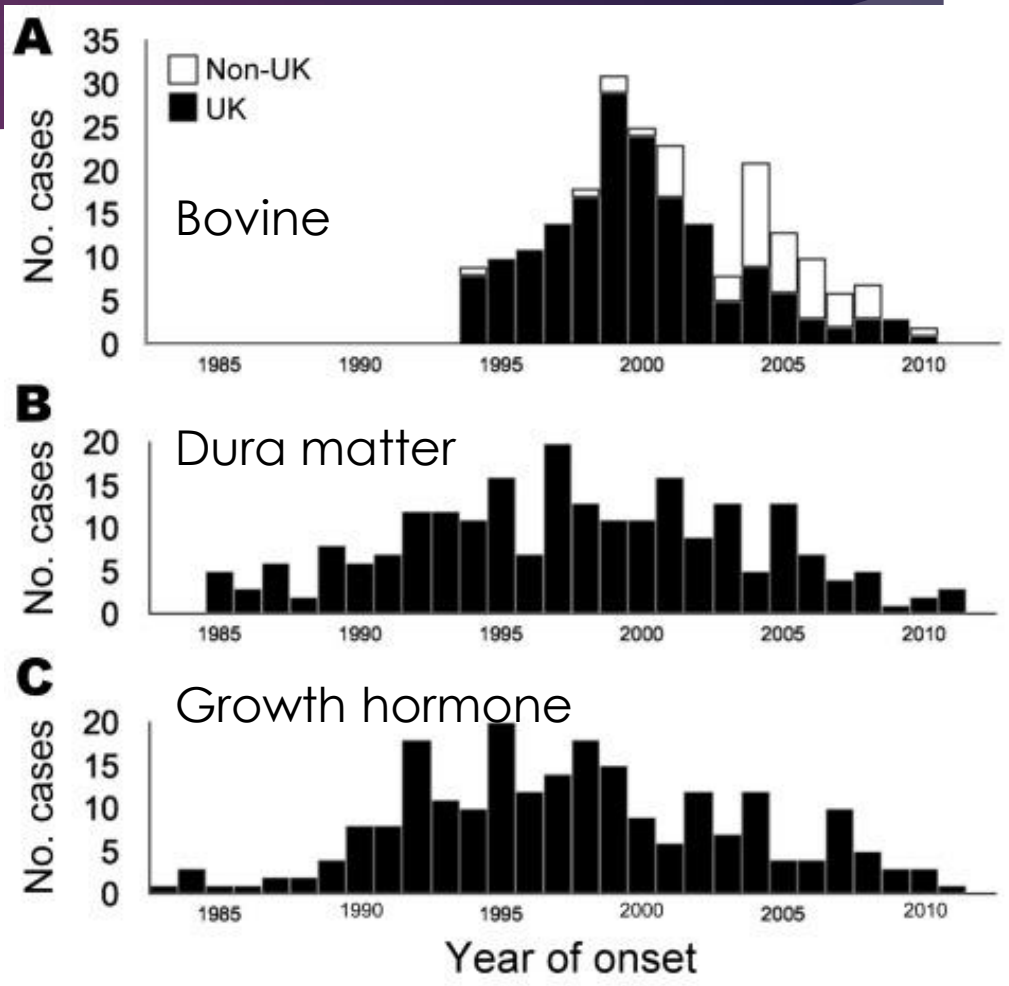
Review of Iatrogentic (acquired) Prion Disease

Global review (2012)

Source of Infection	No. cases	Mean incubation period, y (range)	Clinical signs†
Dura mater graft	228	12 (1.3–30)	Cerebellar, visual, dementia
Neurosurgical instruments	4	1.4 (1–2.3)	Visual, dementia, cerebellar
Stereotactic EEG needles	2	1.3, 1.7	Dementia, cerebellar
Corneal transplant	2	1.5, 27	Dementia, cerebellar
Growth hormone	226	17 (5–42)‡	Cerebellar
Gonadotropin	4	13.5 (12–16)	Cerebellar
Packed red blood cells§	3	6.5, 7.8, 8.3	Psychiatric, sensory, dementia, cerebellar

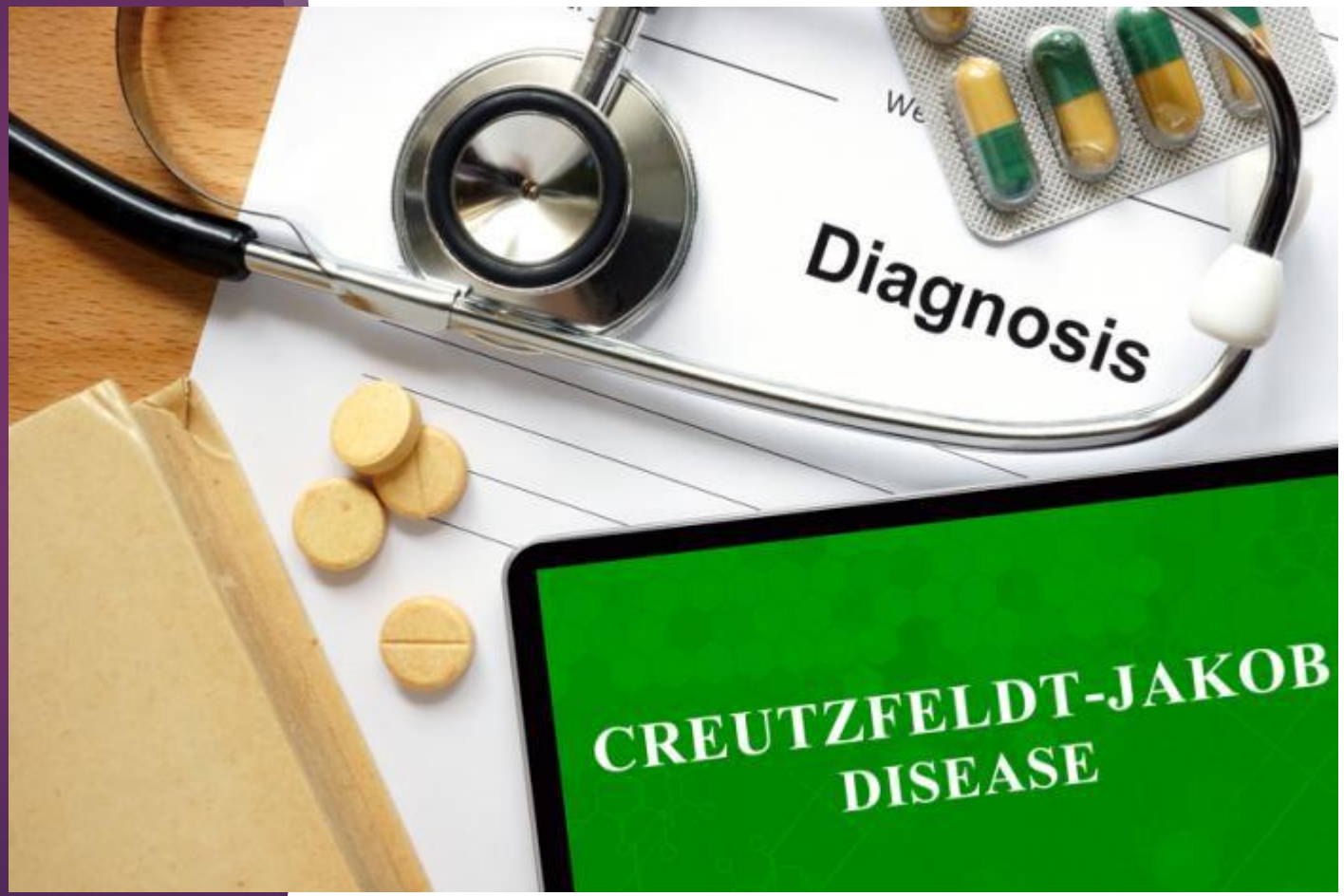
Mostly Japan

Mostly France



https://pubmed.ncbi.nlm.nih.gov/22607808/

Diagnosis



CJD Diagnosis- Clinical Criteria

At least 2 of 4 criteria is Probable for disease

Myoclonus, visual or cerebellar signs, pyramidal or extrapyramidal signs, and (iv) akinetic mutism plus . . .

(i) periodic sharp-wave complexes (PSWCs) on EEG

(ii) a positive 14-3-3 test for the CSF in a patient with disease for less than 2 years

(iii) diffusion-weighted imaging (DWI) or FLAIR abnormalities of the caudate and putamen and/or at least two cortical regions (excluding the frontal cortex)

(iv) No alternative diagnosis

https://journals.asm.org/doi/10.1128/jcm.00769-19

vCJD- Diagnostic criteria

Suspected Variant CJD:

- Current age or age at death less than 55 years
- Psychiatric symptoms at illness onset and/or persistent painful sensory symptoms (frank pain and/or dysesthesia)
- Dementia, and development ≥4 months after illness onset of at least two of the following five neurologic symptoms: impairment in coordination, myoclonus, chorea, hyperreflexia, or visual signs
- Duration of illness of more than 6 months
- Routine investigations of the patient do not suggest an alternative, non-CJD diagnosis
- No history of receipt of cadaveric human pituitary growth hormone or a dura mater graft
- No history of CJD in a first degree relative or prion protein gene mutation in the patient

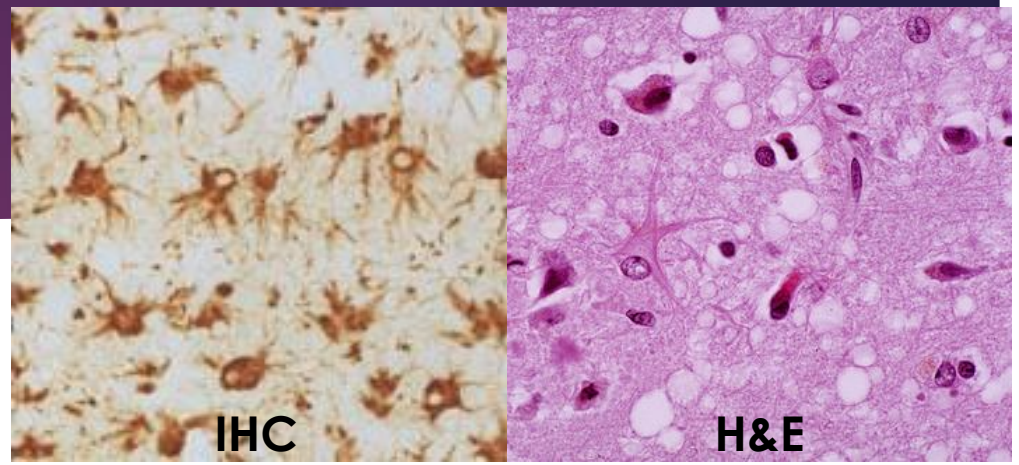
https://pmc.ncbi.nlm.nih.gov/articles/PMC8534461/

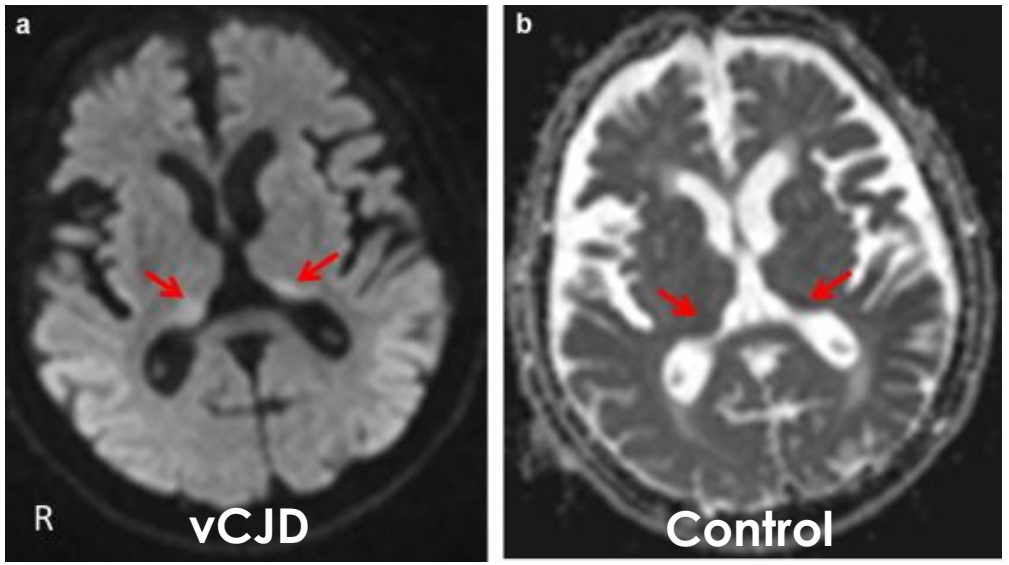
Diagnosis

Definitive:

- Histology of brain biopsy
  - Spongiform encephalopathy
  - PrP^{Sc} positive by staining

- vCJD: tonsil biopsy along with MRI lacking bilateral pulvinar high signal





https://pmc.ncbi.nlm.nih.gov/articles/PMC8534461/

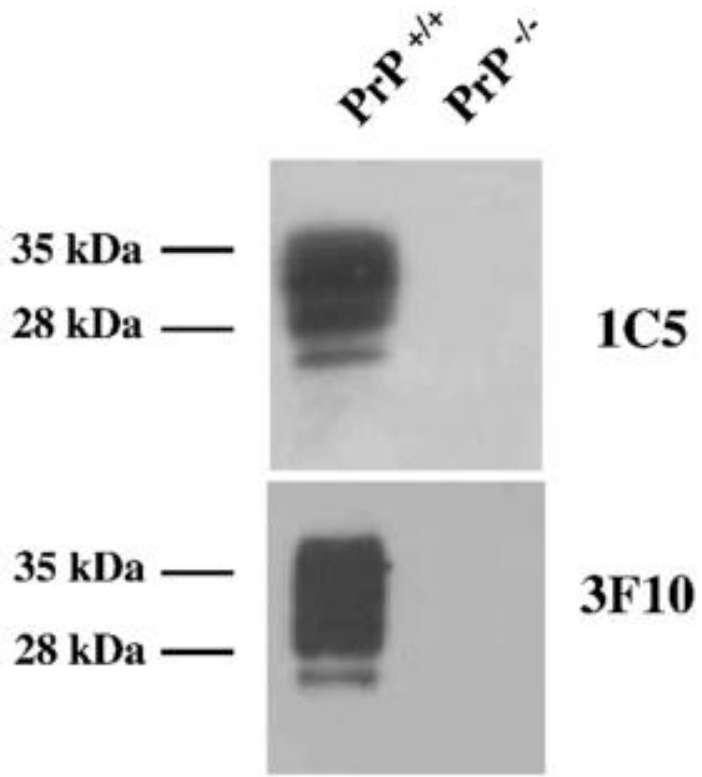
https://www.pnas.org/doi/epdf/10.1073/pnas.95.23.13363

Diagnostic Tests

Western Blot of brain biopsy

- Protease K treatment to destroy normal PrP^{C}
- Western Blot detects only remaining PrP^{Sc}
- Does not detect all types of CJD
  - Variably Protease-Sensitive Prionopath (VPSPr)

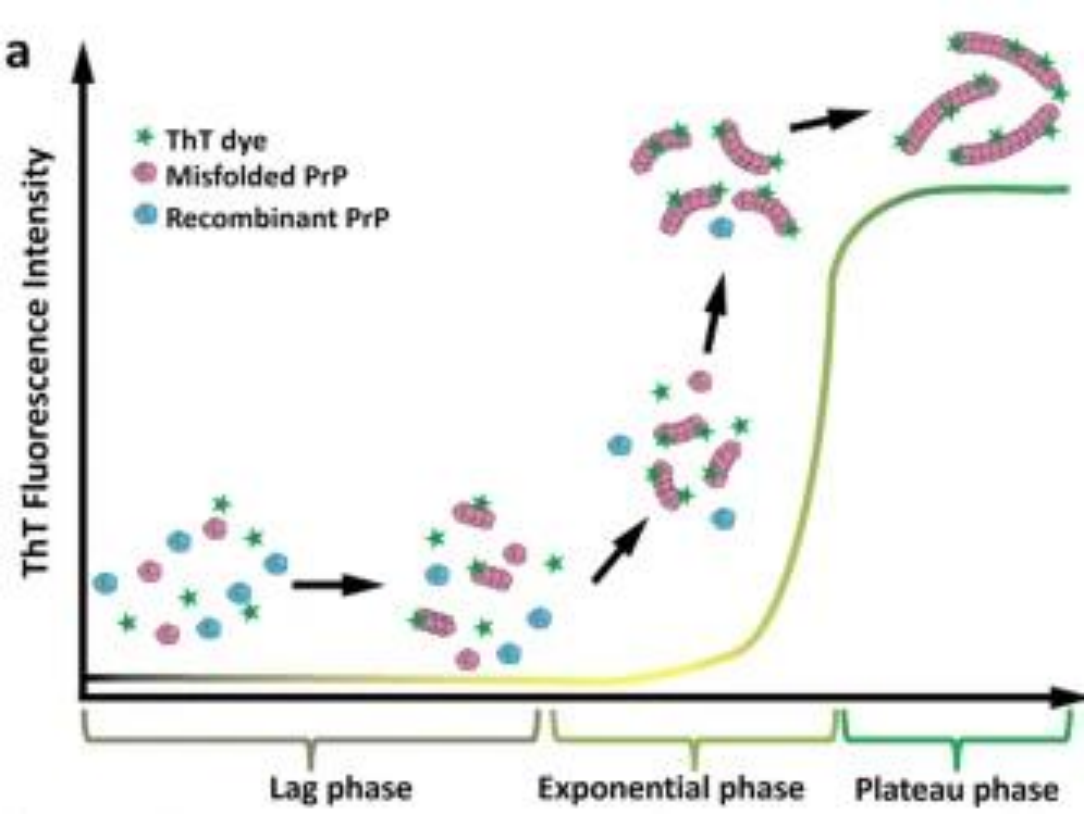
*Takes advantage of the highly stable nature of the mis-folded Prion



Diagnostic Tests

Real-time quaking-induced conversion (RT-QuIC)

- Ultrasonic stimulation of normal PrP^{C} exposed to patient CSF
- Results in plaque like formations of PrP^{Sc}
- Works best for vCJD



https://pmc.ncbi.nlm.nih.gov/articles/PMC3226039/

https://www.mayocliniclabs.com/test-catalog/Overview/620307#Clinical-and-Interpretive

Difference between CJD and vCJD

Characteristic	vCJD, U.K.	Classic CJD, U.S.
Median age at death (years)	28 (range, 14–74)	68 (range, 23–97)[b]
Median illness duration (months)	13–14	4–5
Clinical presentation	Prominent psychiatric/ behavioral symptoms, painful sensory symptoms, delayed neurologic signs	Dementia, early neurologic signs
Periodic sharp waves on EEG	Absent	Often present
"Pulvinar sign" on MRI[c]	Present in >75% of cases	Very rare or absent
Presence of "florid plaques" on neuropathology	Present in great numbers	Rare or absent
Immunohistochemical analysis of brain tissue	Marked accumulation of PrP-res[d]	Variable accumulation
Presence of agent in lymphoid tissue	Readily detected	Not readily detected
Increased glycoform ratio on immunoblot analysis of PrP-res	Present	Not present
Genotype at codon 129 of prion protein	Methionine/methionine[e]	Polymorphic

https://www.cdc.gov/prions/pdfs/public-health-impact.pdf

Diagnostics Gap

- No donor screening tests yet

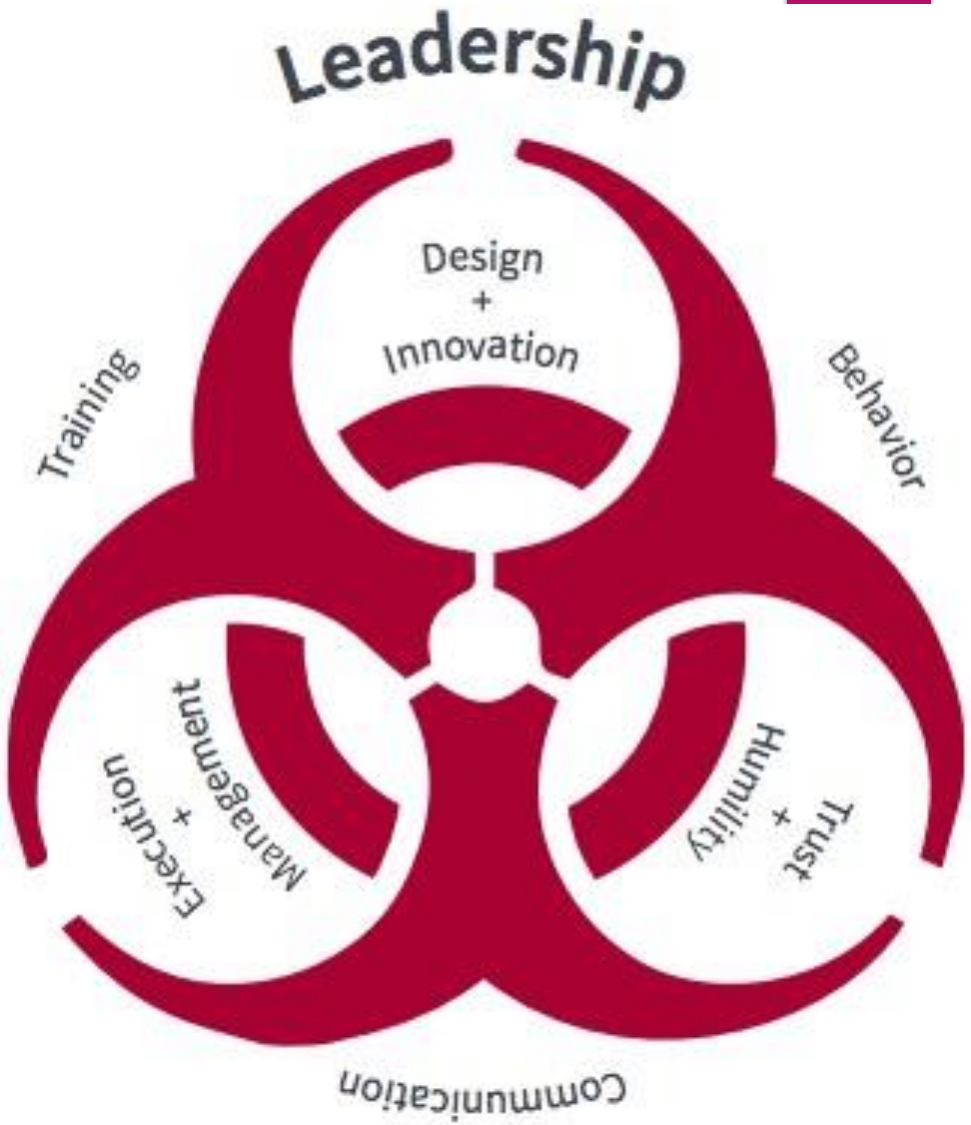
TRANSFUSION MEDICINE | OCTOBER 31, 2024

A diagnostic blood test for prion diseases

Luisa Gregori

- Ban on donations from people in the UK during the BSE epidemic with neurologic symptoms

Biosafety


Leadership
Design
+
Innovation
Behavior
Trust
+
Humility
Communication
Execution
+
Management
Training

Case

- 85 year old male
- Seizures, abnormal brain MRI, acute encephalopathy
- Serum and CSF were submitted for Arbovirus testing
- Requisition was marked with “PRION” and “suspected CJD”

“What additional precautions should we take?”

PrP^{Sc} concentration in different tissues

- Hamsters with Scrapie
  - Brain- 2,300,000 fg
  - Spleen- 2000 fg
  - Buffy coat- 260 fg
  - Plasma- 13 fg
  - Urine- 0.2 fg

https://pubmed.ncbi.nlm.nih.gov/20512142/

Risk by Specimen Type

Infectious Risk	Tissue
High	Brain (including dura mater), spinal cord, posterior eye, pituitary tissue
Low	Spleen, liver, lymph node, kidney, lung, placenta, cerebrospinal fluid, olfactory epithelium
Very Low	Peripheral nerve, intestine, bone marrow, whole blood, leukocytes, serum, thyroid gland, adrenal gland, heart, skeletal muscle, adipose tissue, gingiva, prostate, testis, tears, saliva, sputum, urine, feces, semen, vaginal secretions, milk, sweat

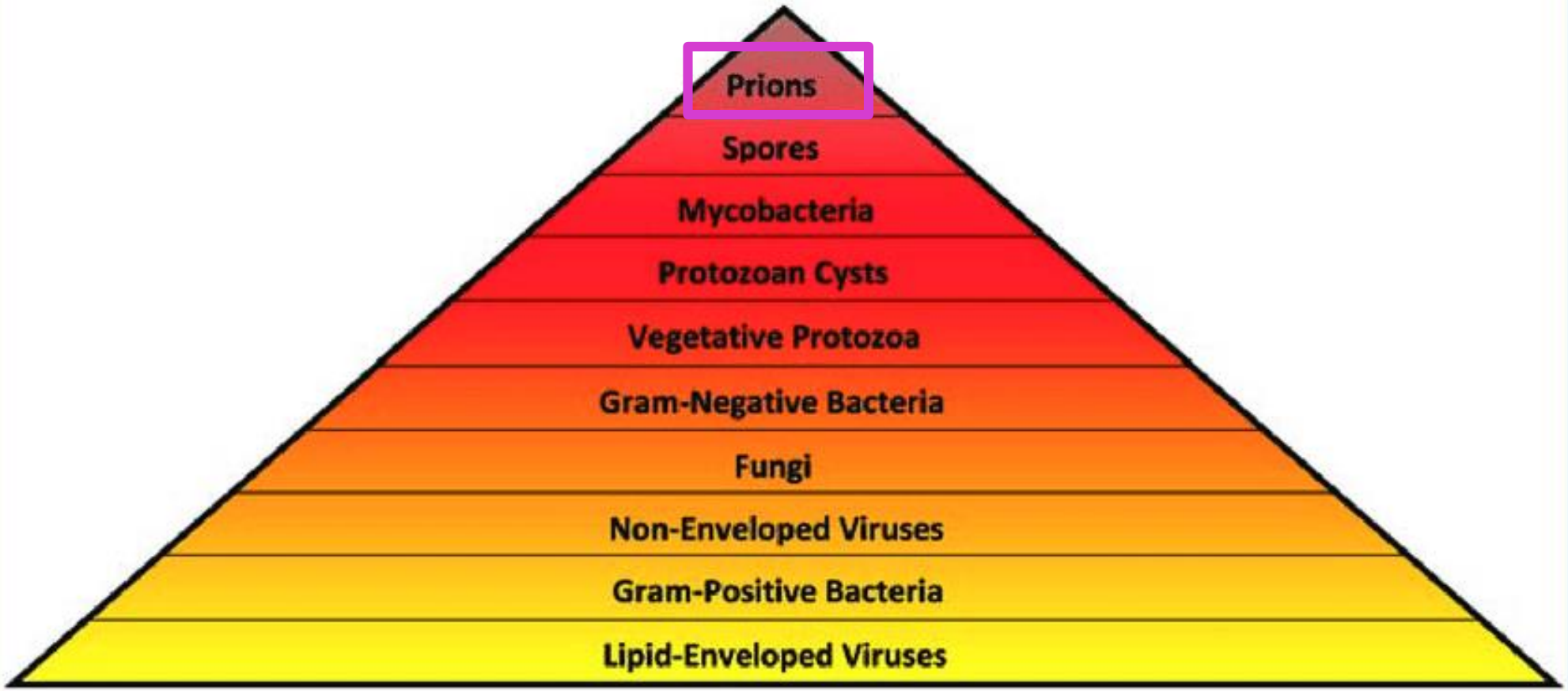
Rutala WA, Weber DJ. 2010. Guideline for disinfection and sterilization of prion-contaminated medical instruments.

Some Biosafety is Standard

*Clinical Lab Recommendations

- Universal precautions: Lab coat, safety glasses, gloves
- BSL2 practices
- Routine medical waste disposal
- Use disposable bench sheets

Most resistant to decontamination/sterilisation



Least resistant to decontamination/sterilisation

Decontamination

1. 1 hour wet contact time or wiping of surface with either :

- 1 N Sodium Hydroxide (NaOH)
  - 40 grams NaOH per liter of water, prepare fresh

OR

- 2% or 20,000 ppm sodium hypochlorite (Bleach)
  - 1:4 dilution of concentrated Bleach (8.8%)
  - 1:2 dilution of household bleach (ranges from 3.25-6.25%)

2. Follow with a distilled water rinse to reduce corrosion

3. Autoclave after rinsing if possible (121°C for 1 hour)

- If unable to autoclave, double NaOH concentration (no change to bleach)

* No EPA or FDA approved product for decontaminating, disinfecting, or sterilizing prions.

*Infectivity is strongly stabilized by drying or fixation with alcohol, formalin or glutaraldehyde. **Keep wet** until decontaminated.

Alternate Disinfection Recommendations

- **Option 1**. Autoclave at 134°C for 18 minutes in a pre-vacuum sterilizer.
- **Option 2**. Autoclave at 132°C for 1 hour in a gravity displacement sterilizer.

Rutala WA, Weber DJ. 2010. Guideline for disinfection and sterilization of prion-contaminated medical instruments.

Disinfection

- Fragile instruments such as endoscopes and electrodes remain a challenge, but new and gentler methods— alkaline cleaning solutions, phenolics, and gaseous hydrogen peroxide—have proven harmless to instruments and give a high, if not always complete, degree of prion inactivation.
- Cover parts of instruments to protect them
- Liquid waste can be collected in a 4 L waste bottle initially containing 600 ml 6 N NaOH, dispose of after 1 hour.

Rutala WA, Weber DJ. 2010. Guideline for disinfection and sterilization of prion-contaminated medical instruments.

Our CJD Suspect

- Much of the assay was able to be moved into a BSC, so staff chose to work there.
- Standard PPE and waste disposal
- No spills or concerns for splashes
- Staff used concentrated bleach and water to clean the BSC after use
- Arbovirus testing was negative

Summary

- Prion disease can occur from random mutations, consumption of prions, exposure during surgical procedures, or it can be inherited.
- Incubation can take many years but neurodegenerative disease is progressive and uniformally fatal, usually within 2 years.
- There are no proven treatments or vaccines
- Diagnosis is usually clinical or post-mortem.
  - Some new methods can detect vCJD with less invasive methods
- Prions are highly stable and require extensive disinfection practices

Resources

- Rutala WA, Weber DJ, Society for Healthcare Epidemiology of America. 2010. Guideline for disinfection and sterilization of prion-contaminated medical instruments. *Infect Control Hosp Epidemiol* 31:107–117.
- WHO: https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/norms-and-standards/transmissible-spongiform-encephalopathies
- CDC: https://www.cdc.gov/prions/about/index.html