

LECTURE 25: PRION DISEASES

Russ Fraser, Fall 2023

Novel Proteinaceous Infectious Particles Cause Scrapie

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emphasize this feature. In place of such terms as "unconventional virus" or "unusual slow virus-like agent," the term "prion" (pronounced *pree-on*) is suggested. Prions are small *pro*teinaceous *in*fectious particles which are resistant to inactivation by most procedures that modify nucleic acids. The term "prion"

SCIENCE, VOL. 216, 9 APRIL 1982

Prions: characteristics

- Non-viral agent
- An abnormal form of a normal protein
  - Normal prion protein (PrP) found in neurons, lymphocytes, and dendritic cells
  - Function is unknown (copper transport?)
- Has unusual properties:
  - Very resistant to inactivation by heat, UV irradiation, γ-irradiation, & chemicals that destroy or modify nucleic acids.
  - Susceptible to procedures that hydrolyze, modify, or denature proteins.
  - Do not induce inflammatory reaction or host immune response in affected animal; affected animals are afebrile.

Prions: characteristics

FEATURE	PRIONS	VIRUSES
Contain nucleic acid	No	Yes
Contain protein	Yes, encoded by cellular genes	Yes, encoded by viral genes
Resistant to heat inactivation	**Yes**	**No**
Appearance by electron microscopy	Amyloid-like	Icosahedral or helical or complex
Infectious	Yes	Yes
Induce antibody response in infected animal	No	Yes
Induce inflammatory response in infected animal	No	Yes

Prions: characteristics

TABLE 31.2 Effects of Physical and Chemical Treatments on Scrapie Prion Infectivity[a]

Treatment	Reduction of Infectivity
1 M NaOH	10^{6-8}
Phenol extraction	10^{6}
0.5% sodium hypochlorite	10^{4}
Histopathologic processing	$10^{2.6}$
3% formaldehyde	10^{2}
1% β-propiolactone	10^{1}
Ether extraction	10^{2}
Autoclave 132°C for 90 min	$10^{7.4}$
Autoclave 132°C for 60 min	$10^{6.5}$
Autoclave 121°C for 90 min	$10^{5.6}$
Boiling 100°C for 60 min	$10^{3.4}$
Heating 80°C for 60 min	10^{1}

[a]*Composite of several studies, therefore no untreated control value given.*

For reference only

From Fenner's Veterinary Virology, 5th ed. Edited by N.J. MacLachlan & E.J. Dubovi (2017), Table 31.2, pg 560.

Prion: the infectious prion hypothesis

- The infectious protein (abnormal prion or prion protein-Scrapie (PrP^{Sc})) has abnormal conformation (or abnormal shape).

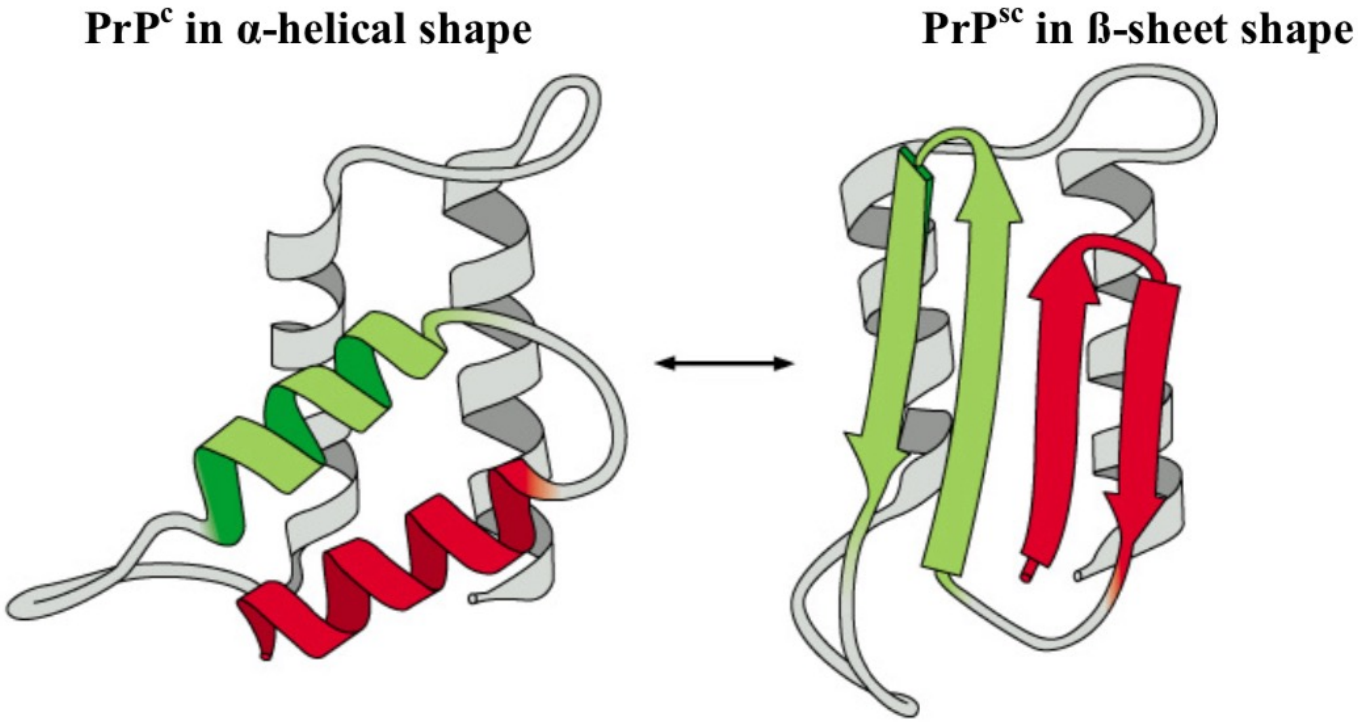
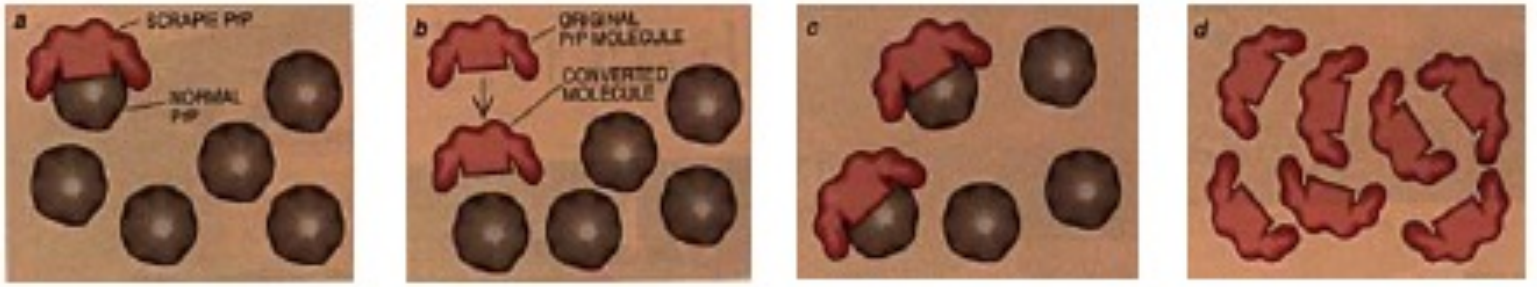


Figure 6–89 Molecular Biology of the Cell, 4th Edition

Prion: the infectious prion hypothesis



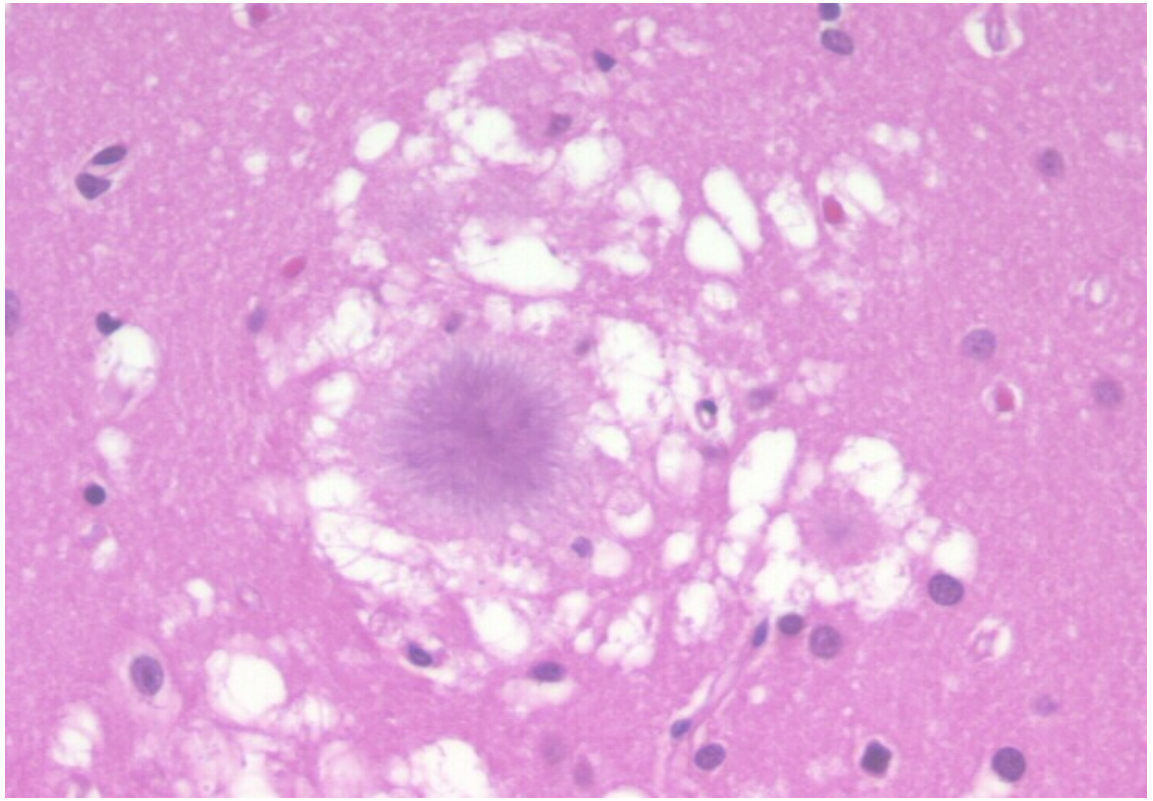
PrP^c gene occurs in all mammals; of unknown function; removal has no ill effects on animal. Mutation in PrP gene results in inherited prion disease.

from, Prusiner, S. 1995. The Prion Diseases. Scientific American.

- It is proposed that when normal cellular prion protein (PrP^C) molecules interact with PrP^{Sc}, they are converted into new PrP^{Sc}.
- So in infectious/transmissible case, PrP^{Sc} is ingested or inoculated → replicates in lymphoid system (Peyers patches? follicular dendritic cells?) → gains access to CNS and continues to convert PrP^C to PrP^{Sc} in suitable locations.

Prions: characteristics

- Affect the CNS
  - Clinical signs are nervous: paralysis, dementia, loss of motor control
- Very long incubation periods
  - 2-5 yrs for scrapie
  - 4-6 years for BSE
- They are 100 % fatal.
- Hallmark is the presence of microscopic vacuolation and accumulation of PrP^{Sc} in the brain



Prions: diseases

TABLE 31.1 Prion Diseases of Animals and Humans

Disease	Host	Source of Infection
Scrapie	Sheep, goats	Uncertain, most likely by direct contact and contamination of pastures by placentas, blood, saliva, and feces
Bovine spongiform encephalopathy	Cattle	Bovine spongiform encephalopathy prion contamination of meat-and-bone meal
Transmissible mink encephalopathy	Mink	Prion contamination of unknown origin fed to mink
Chronic wasting disease	Mule deer, Rocky mountain elk white-tailed deer, black-tailed deer, moose	Unknown source; highly transmissible among deer or elk held in close contact Saliva, feces, blood, and urine contain infectious prions; environmental contamination may play a role in transmission
Feline spongiform encephalopathy	Domestic cats, lions, tigers, puma, ocelot, cheetahs	Bovine spongiform encephalopathy prion contamination of meat fed to animals
Exotic ungulate spongiform encephalopathy	Greater kudu, nyala, oryx, and others in zoos	Bovine spongiform encephalopathy prion contamination of meat-and-bone meal
Atypical scrapie and bovine spongiform encephalopathy	Sheep, goats, cattle	Recently reported disorders, sporadic origin
Kuru	Humans	Ritual cannibalism in Fore people of Papua New Guinea likely initiated by an initial prion-infected individual
Creutzfeldt–Jakob disease	Humans	Iatrogenic: Human prion contamination of dura mater grafts, therapeutic hormones, derived from cadavers; blood transfusion
		Familial: Germ line mutation in prion gene (*PRNP*)
		Sporadic: Unknown cause, perhaps somatic mutation in *PRNP* gene or spontaneous conversion of PrP^{C} into PrP^{Sc}
Variant Creutzfeldt–Jakob disease	Humans	Transmission of bovine spongiform encephalopathy prion to humans, probably by the oral route
Gerstmann–Sträussler–Scheinker syndrome	Humans	Familial: Germ line mutation in *PRNP* gene
Fatal familial insomnia	Humans	Familial: Germ line mutation in *PRNP* gene

PrP, prion protein; PrP^{C}, normal cellular isoform of the prion protein; PrP^{sc}, scrapie isoform of the prion protein.

Modified from Fenner's Veterinary Virology, 5th ed. Edited by N.J. MacLachlan & E.J. Dubovi (2017), Table 31.1, pg 559.


Scrapie

Scrapie

Classical

Naturally transmissible

Atypical

Not naturally transmissible

Sporadic

Classical scrapie

- Most common prion disease caused by PrP^{Sc}
- Not highly contagious (in chronically infected herds, <5% of animals are symptomatic at any one time)
- Host range: small ruminants (sheep and goats)
  - Genetic susceptibility: different genotypes have different susceptibility to scrapie → breeding programs → ↓ incidence
  - Genetic susceptibilities exist in goats, but there is considerably more variation resulting in more difficulty with universal breeding programs.
- Geographic distribution: endemic in all countries
  - Except Australia, New Zealand, & South Africa.
- Incubation period: at least 1 year, usually 2 to 5 years

Classical scrapie: transmission

- Placenta and birth fluids, milk, urine, blood, saliva and feces all contain PrP^{Sc}
- Can be transmitted vertically *in utero*, during parturition and up to weaning
- Horizontal transmission is also possible to other sheep/goats
- PrP^{Sc} can exist for years on pastures, buildings, etc.

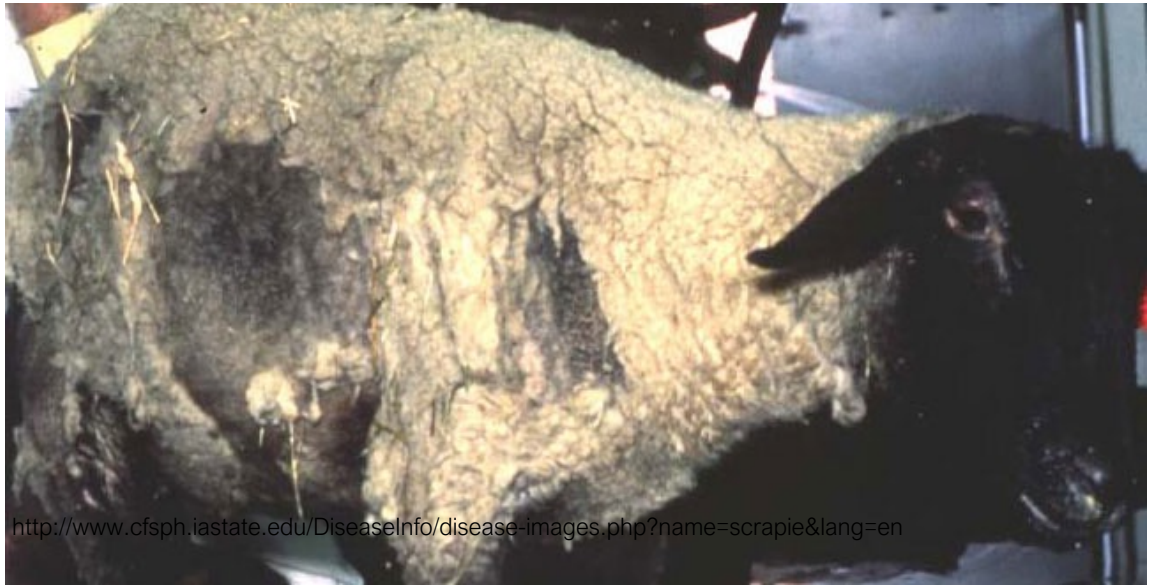


Classical scrapie: clinical signs



Scrapie: clinical signs

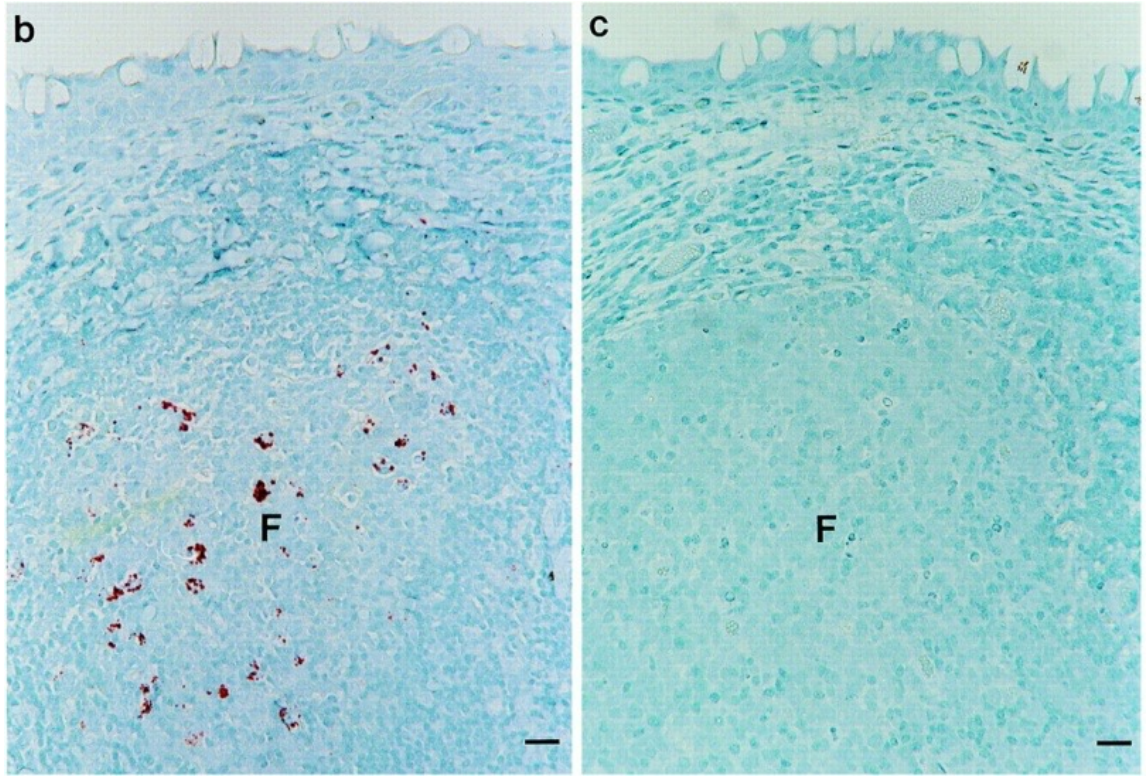
- Often very subtle onset of neurological signs
  - Standing apart, blank stare
- Intense pruritus: animals “scrape” on objects, bite flanks and hind-limbs → alopecia
- Ataxia
  - Usually the only sign in atypical scrapie
- Emaciation
- Hyperesthesia (abnormal sensitivity to normal stimuli)
- Down
- Death


http://www.cfsph.iastate.edu/DiseaseInfo/disease-images.php?name=scrapie&lang=en

Scrapie: diagnosis

- DDx: rabies, visna, & pseudorabies

- Confirmatory diagnosis:
  - IHC on tissue:
    - Brain (post-mortem)
    - Rectal or third eyelid biopsy (ante-mortem)
  - Histopathological exam of the brain for classic lesions:
    - Vacuolation
    - Spongiosis
    - Neuronal degeneration
    - No inflammation



b: scrapie sheep c: normal sheep
Immunohistochemical staining of lymphoid tissue from 3rd eyelid

Scrapie: diagnosis

- DDx: rabies, visna, & pseudorabies

- Confirmatory diagnosis:
  - IHC on tissue:
    - Brain (post-mortem)
    - Rectal or third eyelid biopsy (ante-mortem)
      - A negative result does not guarantee freedom from disease
  - Histopathological exam of the brain for classic lesions:
    - Vacuolation
    - Spongiosis
    - Neuronal degeneration
    - No inflammation

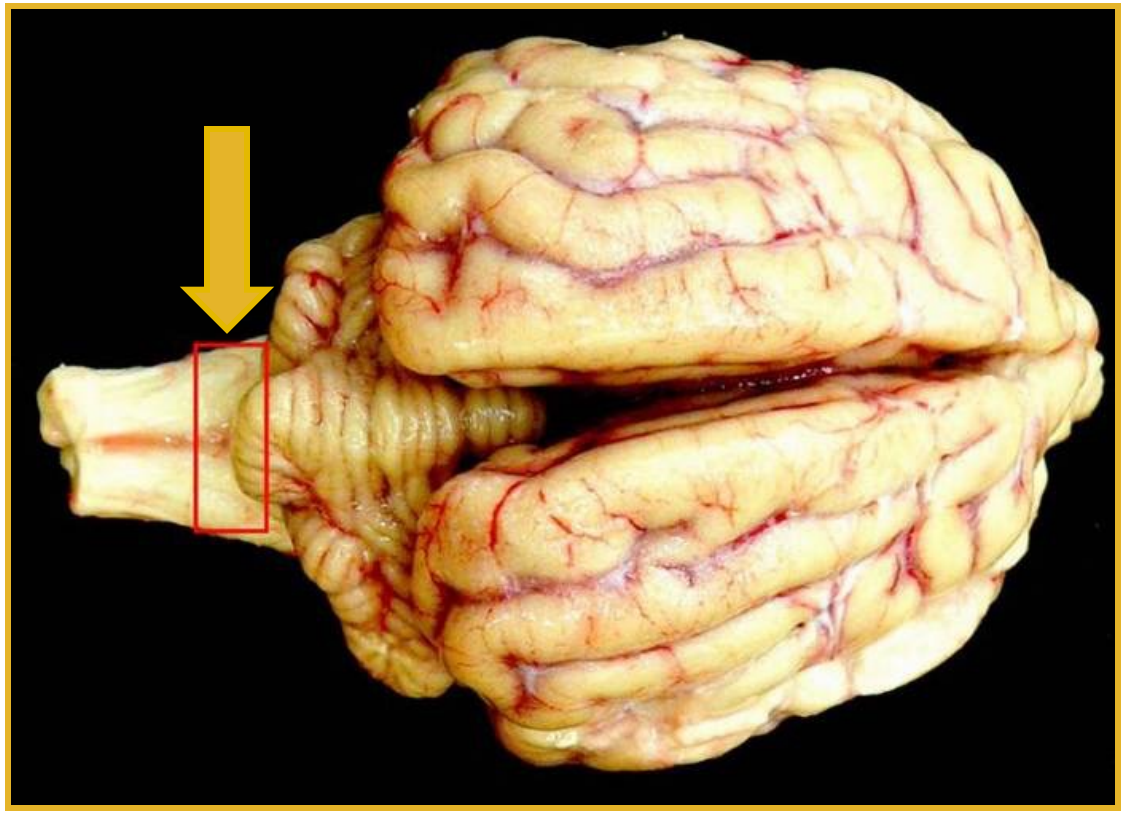


Good restraint and local anaesthesia are needed to collect a sample of lymphoid tissue from the third eyelid. IHC can be performed on this biopsy.

Scrapie: diagnosis

- The red box indicates the region of the obex, which is the portion of the brain that must be obtained for the diagnosis of ALL TSEs





From http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=bovine-spongiform-encephalopathy&lang=en

Scrapie: diagnosis

- TSE rapid tests on brain tissue (i.e., post-mortem sample) for BSE, Scrapie, & CWD, by Bio-Rad Laboratory, and IDEXX Laboratory
- Tests are based on enzymatic digestion of PrP^{c}, leaving the PrP^{Sc} undigested;
- Extremely sensitive
- Give results in about 4 hours
- Now used to screen brain samples in BSE surveillance programs (while carcass is held at abattoir, before releasing beef to public) both in Canada & USA.
- Tissues from animals that test positive will undergo further testing at referral labs.

Scrapie: control

- SCRAPIE IS A REPORTABLE DISEASE

- Canada:
  - Control is by eradication: infected sheep & all animals exposed to the same birthing environment are destroyed & owner is compensated.
    - Genetically resistant animals may be allowed to remain.
  - In absence of reliable individual animal identification & management records, an entire flock is ordered destroyed.
  - Voluntary scrapie flock certification program with surveillance & certification
  - Breeding for resistance is possible for both goats and sheep



Bovine spongiform encephalopathy

BSE, “Mad cow disease”

BSE

Classical

Transmissible

Atypical

Transmissible

Sporadic

BSE: general

- Transmission: feeding meat and bone meal (MBM) to dairy cows.
  - MBM: a high-protein nutritional supplement prepared from offal of sheep, cattle, pig & chicken carcasses.
  - Horizontal transmission is very poor/non-existent
- Long incubation period: 4-6 yrs
- Host range: broad (must consume infected material)
  - Humans
  - Cats, wild ungulates, mink

BSE: clinical signs

- Cattle affected by BSE experience progressive degeneration of the nervous system.
- Affected animals show:
  - Apprehension
  - Changes in temperament, such as nervousness/hyperexcitability or aggression.
  - Abnormal posture (e.g. abnormal head carriage).
  - Pelvic limb ataxia (incoordination and difficulty in rising).
  - Rapid weight loss despite continued appetite.
  - Decreased milk production.



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BSE: diagnosis

- DDx: BVD, IBR, MCF, pseudorabies, & rabies (viral diseases with CNS signs in cattle).
  - Cases of BSE are usually isolated – when multiple animals are affected by neuro disease, BSE → ↓ on the list

- Testing: brain samples only (no ante-mortem test).
  - Screened using rapid tests (enzymatic digestion of PrP^C protein)
    - Any positive or "inconclusive" results are sent to Canada's national BSE reference laboratory in Edmonton for confirmatory testing using IHC.
  - IHC

BSE: control

- Reportable
- Canada:
  - 1990: importation of cattle from the UK and Northern Ireland has been banned
  - 1997: ban of feeding MBM to cattle
  - 2005: Illegal to transport downer cattle, and cattle with neuro signs would be screened out at slaughter
  - 2007: All SRM are banned from pet foods, animal feeds, and fertilizers (as well as food for human consumption)
    - Brain, spinal cord, tonsils, eyes of cattle > 30 months
  - On going surveillance, especially of high risk cattle:
    - Neurological signs
    - > 30 months old
    - Downer, emergency slaughter animals, or animals found sick pre-slaughter

BSE: public health significance

- Disease in cattle resembles Kuru & CJD in humans.

- Zoonotic: vCJD is caused by BSE

If you've lived or spent time in the **United Kingdom, Republic of Ireland or France** during the 'mad cow disease' outbreak of the 1980s and 1990s, **you may now be eligible** to donate blood, plasma and platelets in Canada.



BSE: atypical form

- Extremely rare
- Spontaneous disease in older animals (avg 12 years old, range 5 – 18)
- 3 cases have ever been reported in Canada
- Clinical signs are identical to classical form
- Has potential for transmission via feed
- Control measures are identical to classical BSE



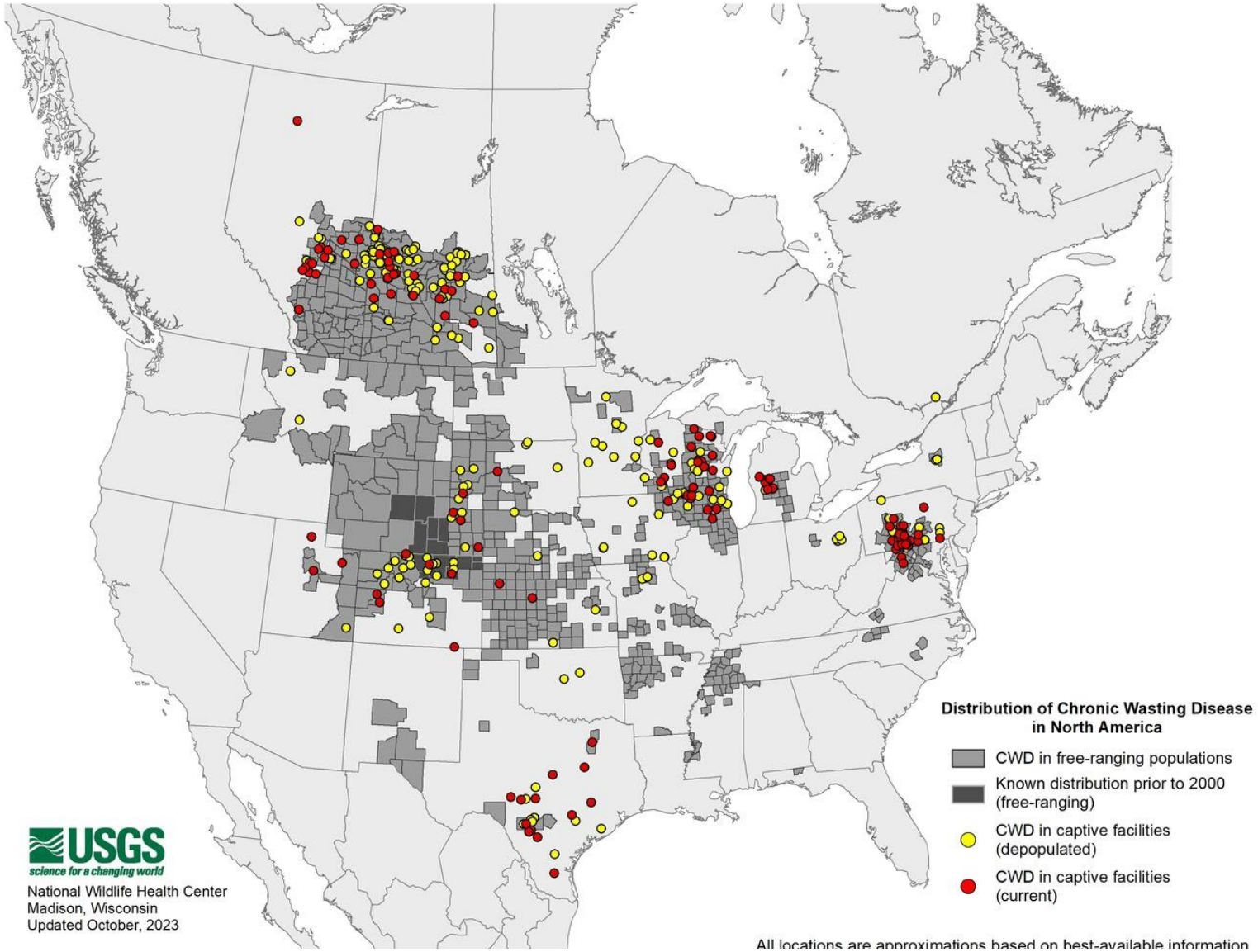
Chronic Wasting Disease

CWD

Chronic Wasting Disease

- CWD is an emerging disease
  - Expanding geographically & increasing in prevalence
- CWD affects free-ranging wildlife & farmed cervid populations
  - Deer
  - Elk
  - Moose
  - Reeindeer
  - Does not affect domestic cattle or sheep
- Incubation period: 16 – 36 months
- CWD has been detected in 31 states and 4 provinces

CWD: distribution



CWD: distribution

Distribution of CWD Globally

Country	Free-ranging cervids	Captive cervids
Canada	Y	Y
Finland	Y	
Norway	Y	
South Korea		Y
Sweden	Y	
United States	Y	Y

CWD: transmission

- Directly between mucous membrane contact
- Indirect:
  - Consumption of contaminated soil, food, water

- Prions are present in blood, tissues, and excretions of sick or dead animals

CWD: clinical signs

- Emaciation/wasting
- Lethargy
- Ataxia
- Excessive salivation
- Loss of prey behavior
- Death

- May be asymptomatic before sudden death



whitetail buck with CWD

CWD: diagnosis

- Challenging antemortem

- IHC on obex postmortem

Currently, CWD tests officially approved by the Canadian Food Inspection Agency (CFIA) are designed for surveillance purposes and are not reliable enough to detect the disease in animals under 12 months of age. CFIA's surveillance testing aims to identify farmed animals over 12 months of age (who are more likely to be infected with CWD), in order to prevent contaminated meat or other consumable products from entering the market. There is currently no test available to certify that food or other consumable products are free from the CWD prion.

CWD: control

- Reportable

- In Canada:
  - All cervids > 1 year slaughtered in (specific provincial) abattoirs are tested
  - Testing of hunted carcasses varies by province
  - Suspected cases are euthanized and tested

- Eradication program failed, has now shifted to one of control and voluntary certification

CWD: public health

- There is some concern that CWD may be transmitted to humans through consumption of deer meat, like with BSE
  - Experimental transmission of CWD to primates and to transgenic mice has been reported
  - To date there is no evidence that CWD poses a risk to humans or non-cervids.
  - As a precaution, the U.S. CDC, CFIA, and WHO recommend not consuming meat from infected animals
  - Hunters are provided with simple precautions to follow when handling any deer, moose or elk.
  - Products from animals infected with CWD are not allowed to enter the human or animal food chain.



https://tpwd.texas.gov/huntwild/hunt/images/feature-hunters-for-the-hungry.jpg

Prions: unique features



Genetic susceptibility/resistance is unique



Zoonotic risk is unique



Only wild animal TSE