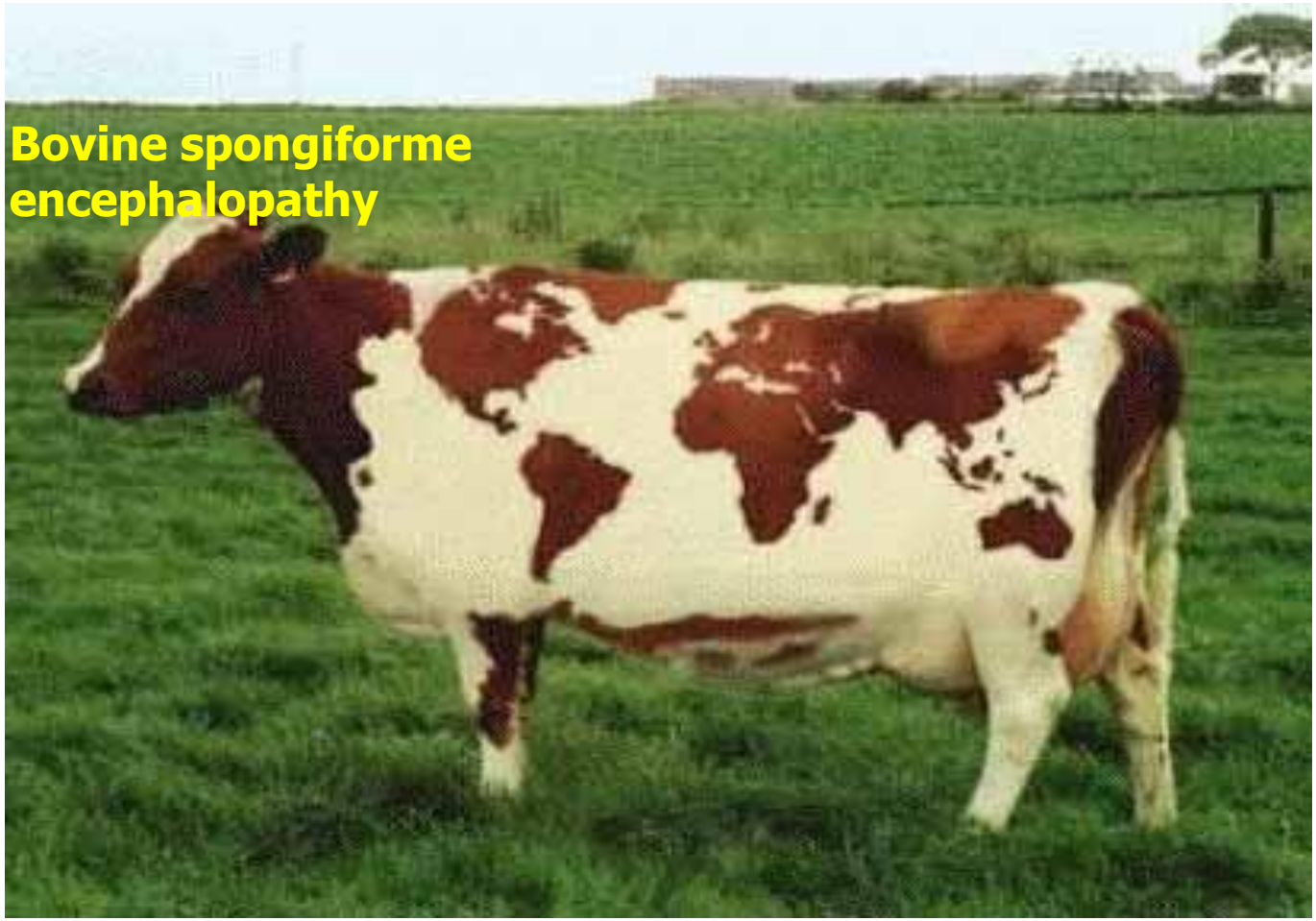
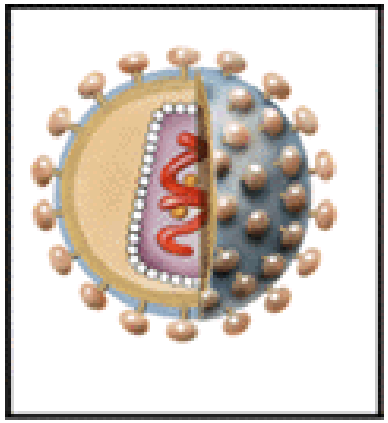
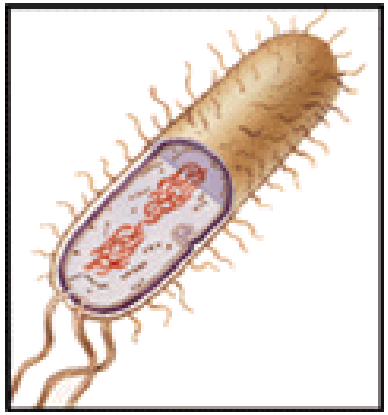
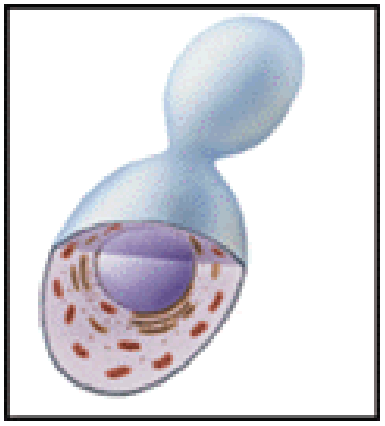
“Global Cow“



Classic causative agents of infectious diseases







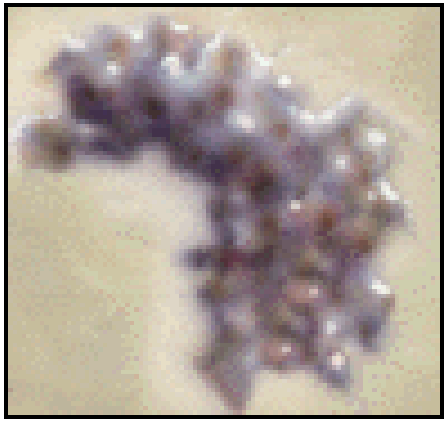
PRIONI

- PRION = ***pro****teinaceous* ***in****fectious particle that lacks nucleic acid*

proin → prion

(Prusiner, 1982)

Characteristics of PrPc / PrPsc



- **PrPc -** soluble protein, part of normal cells, function not known, noninfectious

- **PrPsc** – insoluble protein, pathologic isoform, infectious

Terminology

- **PrPc ili PrPsen**
- c = cellular
- sen = sensitive

- **PrPsc ili PrPres**
- sc = scrapie, TSE form
- res = resistant

Pathologic prions

- Infectious proteins
- No nuleic acid
- Cause neurodegenerative diseases in humans and animals
- Long incubation period (ranije nazivani sporim virusima)
- Disease always fatal

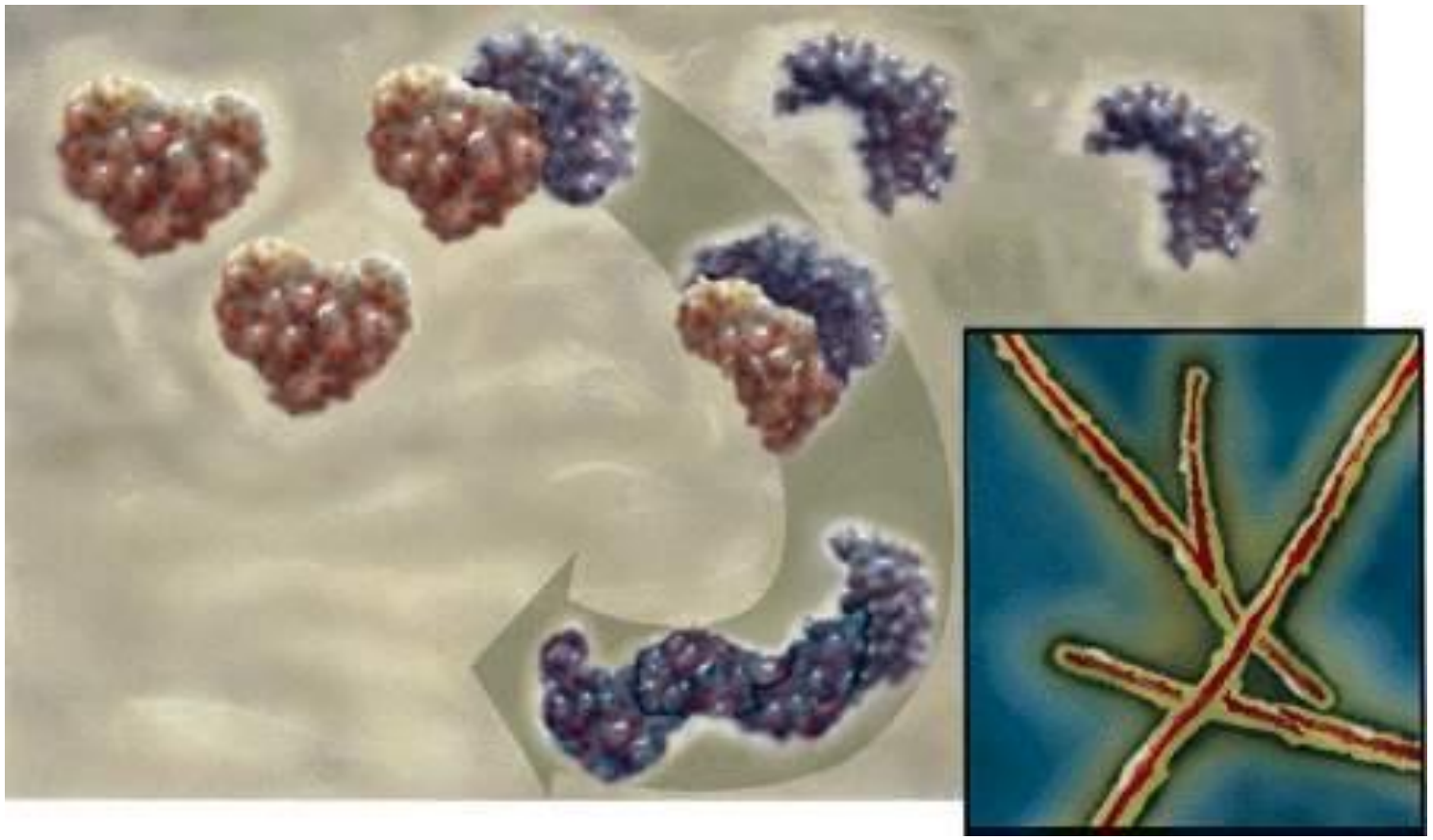
Viruses vs prions

	VIRUSES	PRIONS
Filtrability	yes	yes
Infectivity	yes	yes
Nucleic acid	yes	no
Recognizable shape (el. Microscope)	yes	no
Proteins	yes	no
Size	18-300 nm	4-6 nm

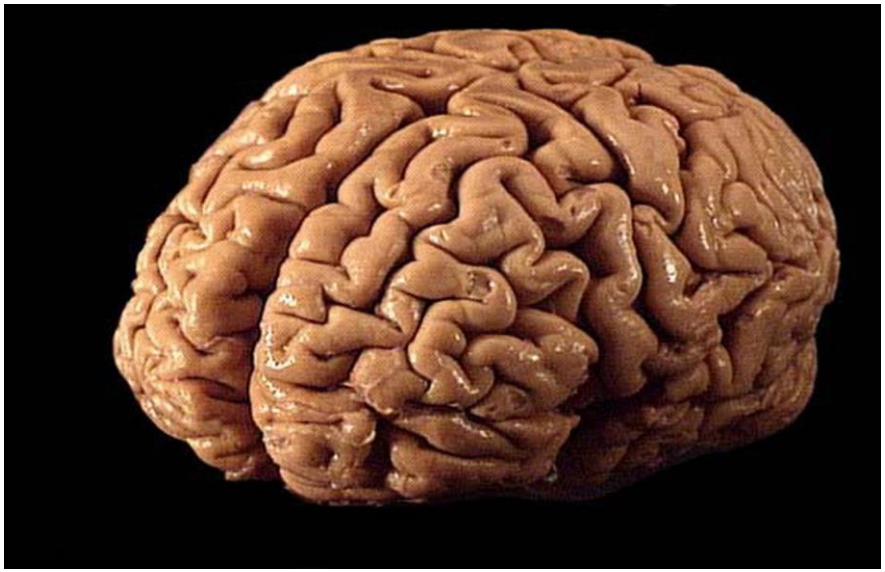
Viruses vs prions

	VIRUSES	PRIONS
CPE in cells	yes	no
Incubation	variable	long
Immune answer	yes	no
Interferon rpoduction	yes	no
Inflamation	Usually yes	no

Patogenesis of prion diseases

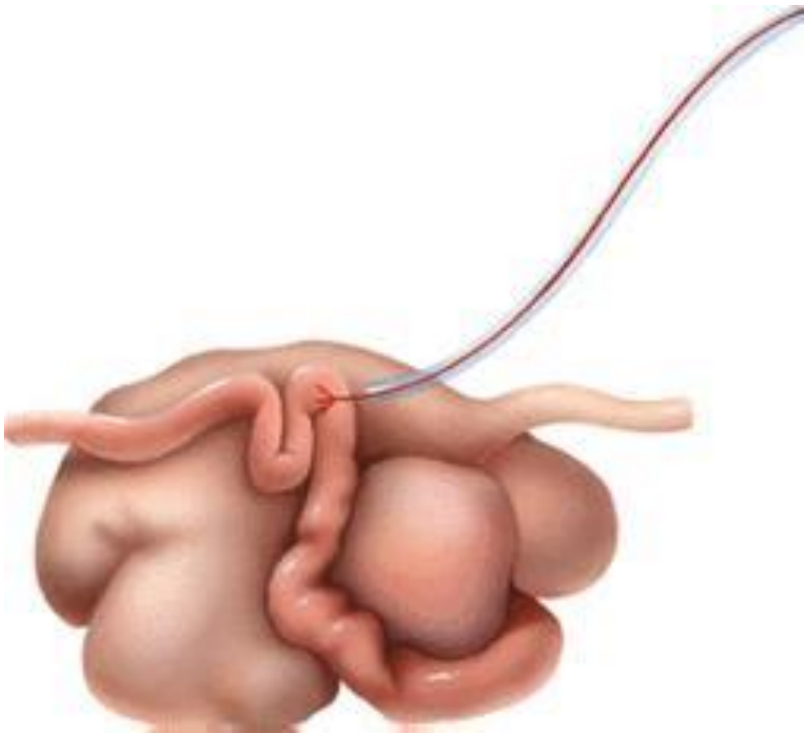


Prions



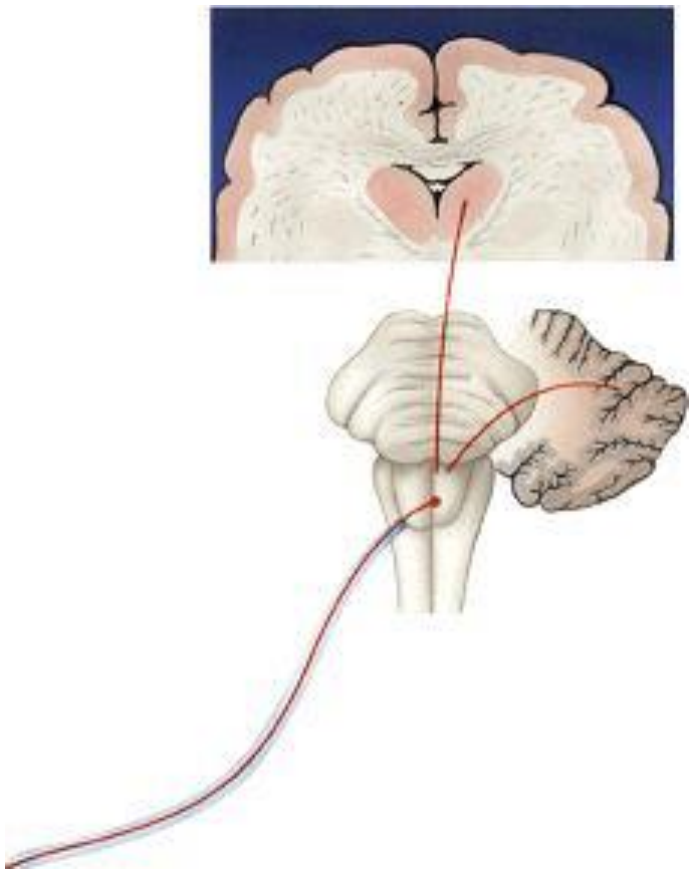
- can be found throughout our bodies and in *high concentrations* in the *brain*

Infection



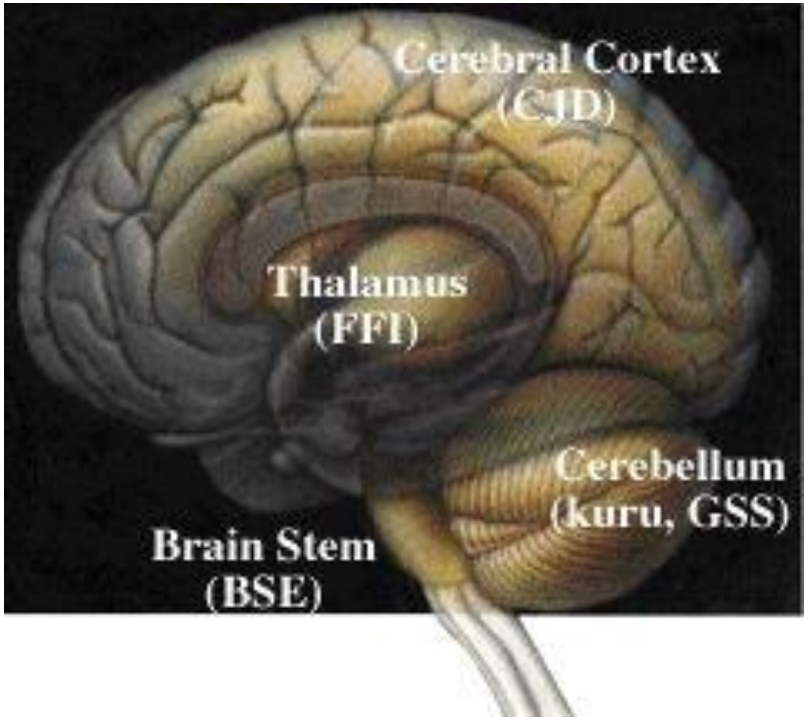
- Ingestion and uptake of pathologic prions
- Neuronal transmission

Propagation and transformation



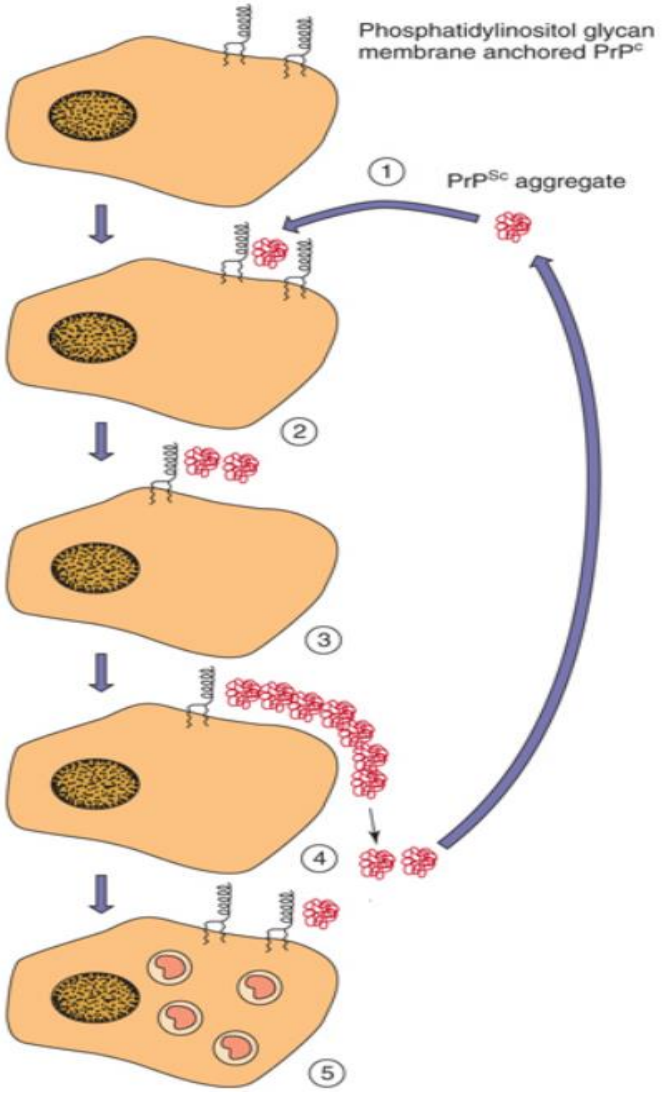
- Accumulation of pathologic prions in brain
- Transformation of “normal“ prions in pathologic

Affinity



- Prioni zahvacaju razne regije mozga
- Spuzvasta degeneracija nastaje odumiranjem zivcanih stanica
- Simptomi ovise o zahvacenoj regiji mozga

Template-mediated protein refolding model for proliferation of prions



- PrPC is a normal cellular protein that is anchored in the cell membrane by phosphatidylinositol glycan.
- PrPSc is a hydrophobic globular protein that aggregates with itself and with PrPC on the cell surface *(1)*.
- PrPC acquires the conformation of PrPSc *(2)*.
- The cell synthesizes new PrPC *(3)*,
- and a chain is built along cell surface anionic glycosaminoglycans *(4)*.
- The chain breaks upon phagocytosis or from shear forces and releases PrPSc aggregates that act like seed crystals to start the cycle over.
- A form of PrPSc is internalized by neuronal cells and accumulates *(5)*.

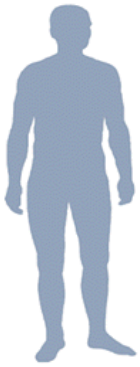
Prion Diseases / Transmissible Spongiform Encephalopathies

- Animal prion diseases
- Human prion diseases

Animal Prion Diseases

Disease	Animal
Scrapie	sheep
Bovine spongiforme encephalopathy	cattle
Transmissible mink encephalopathy	mink
Chronic wasting disease	mule deer, elk
Feline spongiform encephalopathy	cats
Exotic ungulate encephalopathy	kudu, nyala, oryx

BSE

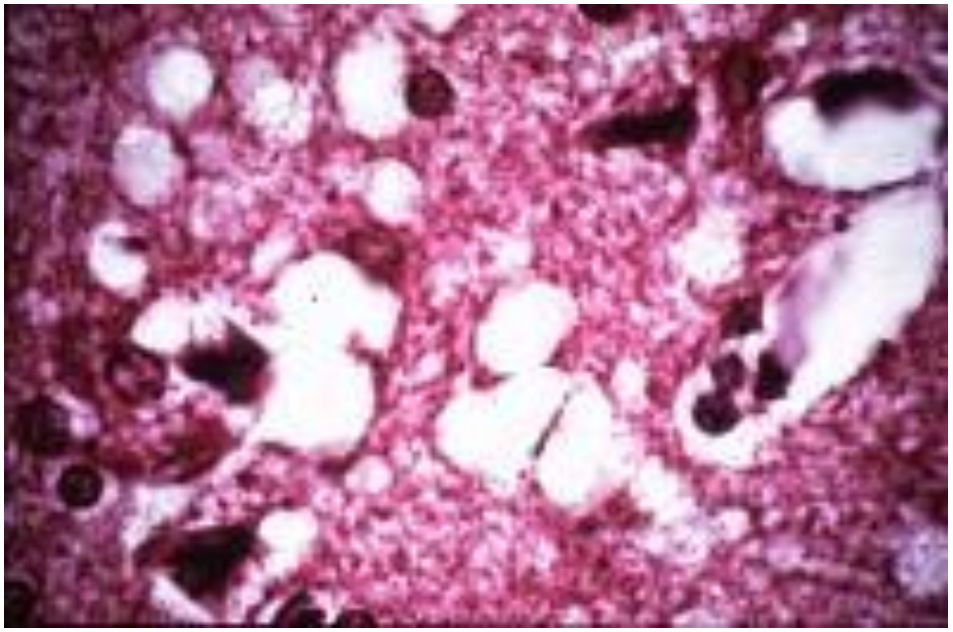


Human Prion Diseases

• Creutzfeldt-Jakob disease	Pathogenesis
– Sporadic CJD	Spontaneous conversion of PrP
– Familial CJD	Germline mutation in PRP gene
– Iatrogenic CJD	Iatrogenic inoculation
– Variant CJD	Infection from bovine prions?
• Fatal familial insomnia	Germline mutation in PrP gene
• Gerstmann-Sträussler-Scheinker disease	Germline mutation of PrP gene
• Kuru	Infection through cannibalism

Kuru

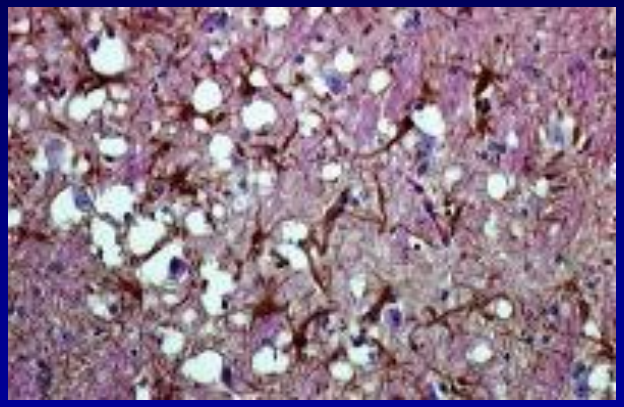
Kuru



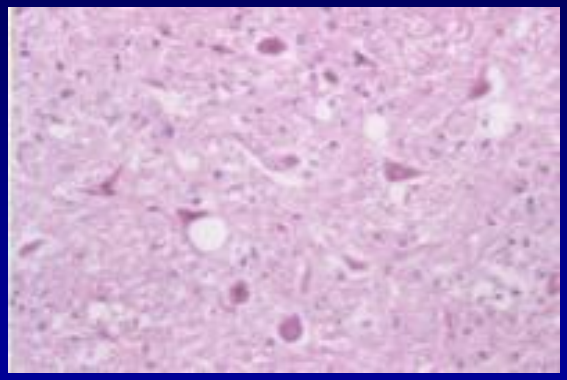
Variant Creutzfeldt-Jakob Disease

- First identified in 1996
- Causally linked to BSE
- Distinct from sporadic CJD
- No germline mutation
- All 109 patients with vCJD have MM genotype at codon 129 (controls 39% and sporadic CJD 79%)
- All bovines have MM genotype at the equivalent site to the human codon 129 polymorphism

Scrapie

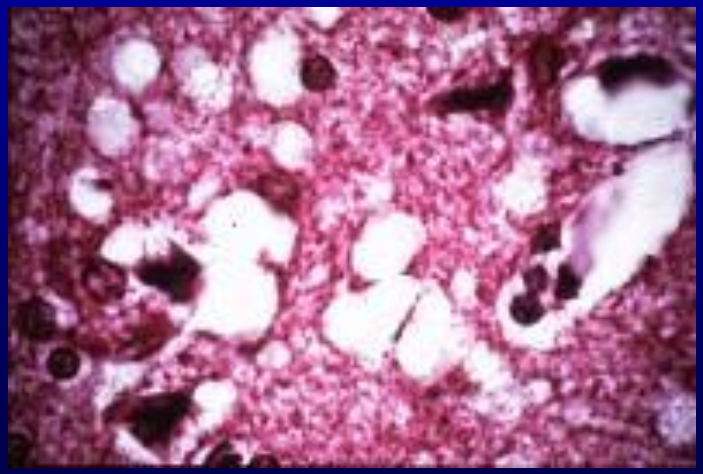


BSE

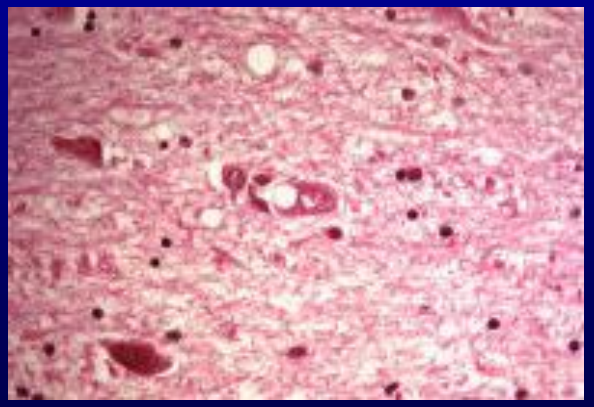


Spongioformne encefalopatije

Kuru



vCJD



Routine diagnostic procedures

- Diagnosis *post mortem*
- Monoclonal antibody based :
  - Immunohistochemistry
  - Immunoblot
  - ELISA
- Molecular biology (predisposition, not prion detetction):
  - DNA Sequencingiranje
  - PCR

Specimen collection


WICHTIGER

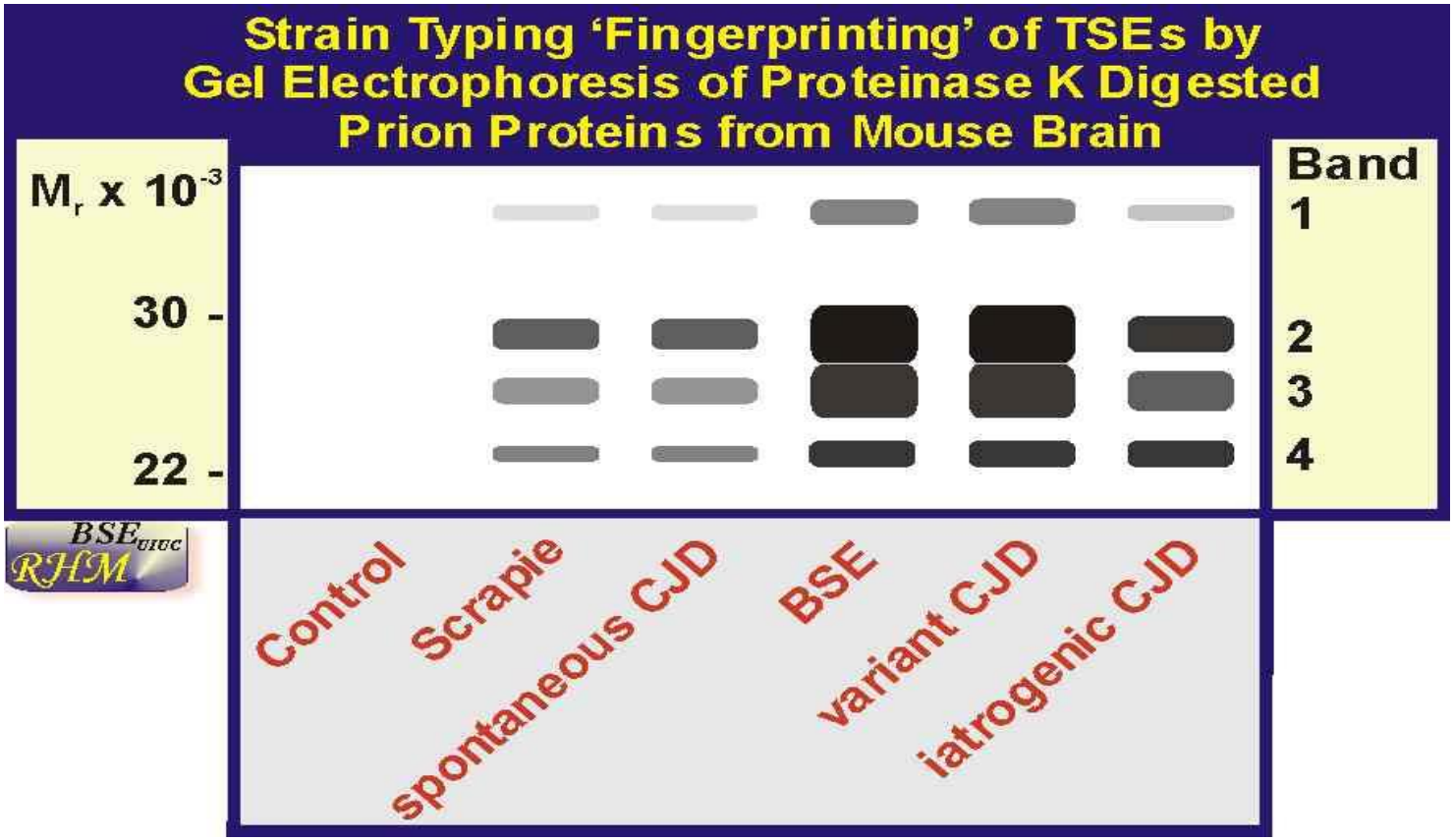
Proteinase K

Homogenisation

Homogenisation

Homogenisation

Western Blot



EIA Test

Sequencing

- **ABI 310** capillary sequencer

Aktuelna specificna dijagnostika prionskih bolesti

- Dijagnoza se postavlja **post mortem**, iznimno nakon biopsije
- Definitivna dijagnoza se bazira na neuropatoloskom nalazu i identifikaciji PrPsc u postmortalnom uzorku
- Ispitivanje prion-gena je metoda izbora u genetski determiniranim prionskim bolestima

Prionske bolesti = prenosive spongioformne encefalopatije

- Prionske bolesti životinja
- Prionske bolesti čovjeka

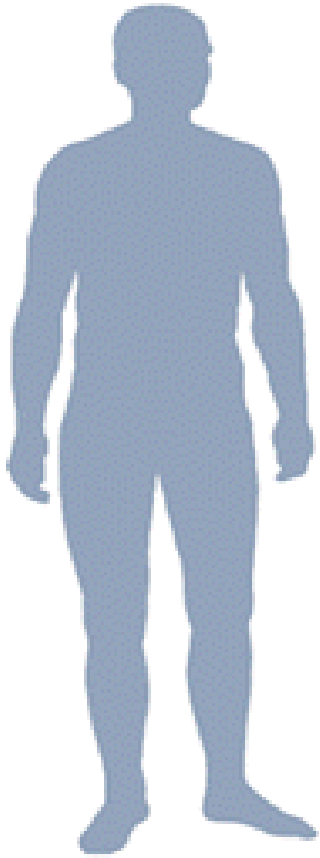
Karakteristike prionskih bolesti

- Dugi inkubacioni period (godine)
- Progresivna, fatalna bolest
- Lezije u mozgu: vakuolizacija, deponiranje materijala sličnog amiloidu
- Nepostojanje upalnog niti imunološkog odgovora
- Uzrokovane nekonvencionalnim agensom, na nekonvencionalan način

Prionske bolesti životinja

Bolest	Vrsta
Scrapie	ovca
Bovine spongiforme encephalopathy	govedo
Transmisivna encefalopatija kune	kuna
Chronic wasting disease	los
Spongiformna encefalopatija mačaka	macka
Egzotična encefalopatija papkara	kudu, nyala, oryx

Prionske bolesti čovjeka



- Spontana pojava
- Jatrogena transmisija
- Mutacija u prion-genu
- Horizontalna transmisija (ingestija, transfuzija??)

Prionske bolesti čovjeka

• Creutzfeldt-Jakobova bolest	Patogeneza
– Sporadična CJD	Spontana konverzija PrP
– Familijarna CJD	Mutacija u PRP genu
– Jatrogena CJD	Iatrogena inokulacija
– Varianta CJD	Infekcija govođim prionima
• Fatalna familiarna insomnia	Mutacija u PrP genu
• Gerstmann-Sträussler-Scheinker disease	Mutacija u PrP genu
• Kuru	Infekcija kanibalizmom

vCJD / BSE
Da li postoji veza?

Varianta Creutzfeldt-Jakobove bolesti

- Prvi put opisana 1996. godine
- Uzročno vezana za BSE
- Razlikuje se od sporadične CJD
- Nema genetske mutacije
- Postoji genetska predispozicija - faktor rizika (svi do sada zabilježeni slučajevi imaju MM genotip na kodonu 129 (kontrole 39% i sporadična CJD 79%)
- Sva goveda imaju MM genotip na mjestu ekvivalentnom ljudskom kodonu 129

Aktuelna dijagnostika prionskih bolesti

- Dijagnoza se postavlja ***post mortem***, iznimno nakon biopsije
- Definitivna dijagnoza se osniva na neuropatološkom nalazu i identifikaciji PrP^{sc} u postmortalnom uzorku (imunohistokemija, imunoblot, ELISA)
- Plazminogen se veže na PrP^{sc}

Prioni

- Infektivni proteini
- Nemaju nukleinske kiseline
- Uzrokuju neurodegenerativne bolesti ljudi i životinja
- Dugi inkubacijski period (ranije nazivani sporim virusima)
- Bolest uvijek fatalna

Usporedba svojstava virusa i priona

	VIRUSI	PRIONI
Filtrabilni	da	da
Infektivni	da	da
Nukleinska kisel.	da	ne ?
Definirani oblik (el. mikroskop)	da	ne
Proteini	da	da
Veličina	18-300 nm	4-6 nm

Usporedba učinka virusa i priona

	VIRUSI	PRIONI
CPE na stanicama	da	ne
Vrijeme inkubacije	ovisi o virusu	dugo
Imuni odgovor	da	ne
Stvaranje interferona	da	ne
Upalni odgovor	obično da	ne

Usporedba osjetljivosti virusa i priona

DEZINFEKCIJA	VIRUSI	PRIONI
formaldehid	da	ne
proteaze	neki da	ne
Grijanje 80°C	većina da	ne
Ionizirajuće zrake	da	ne
UV zrake	da	ne

Svojstva PrP^c priona

- Kodira ih kodon 129 na 20. ljudskom kromosomu
- Solubilan osjetljiv na proteinazu
- Nalazi se na površini glija stanica, B limfocita, neurona CNS-a, keratinocita i brojnih drugih stanica u ljudskom tijelu
- Funkcija mu nije potpuno razjašnjena

Prioni

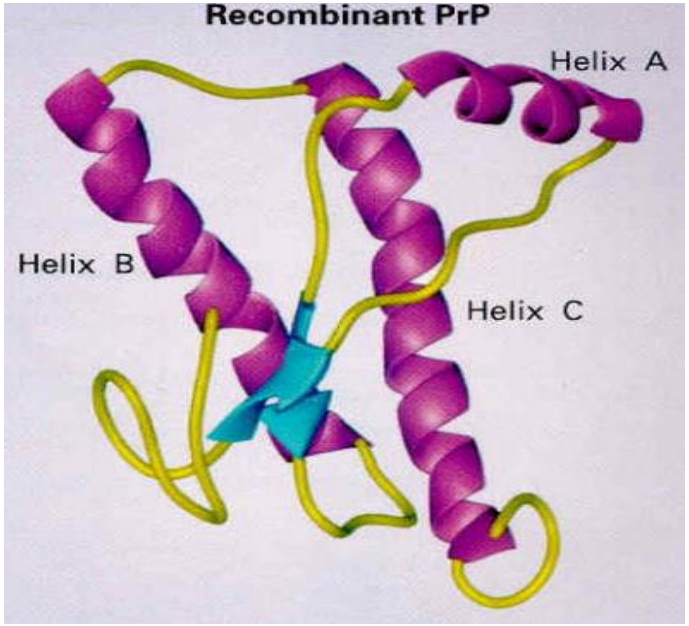
PrP^{c} ili PrP^{sen}

- C = *cellular*, jer je sastavni dio stanica
- sen = osjetljiv, jer ga mogu razgraditi proteinaze

PrP^{sc} ili PrP^{res}

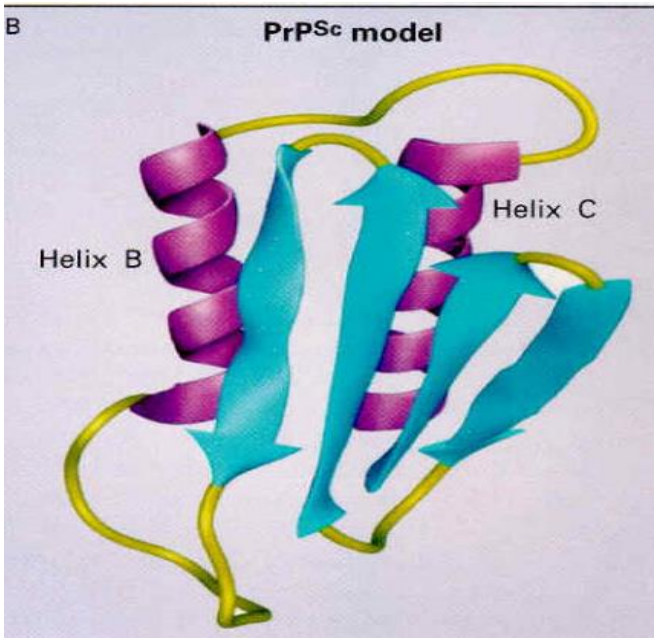
- sc = *scrapie*
- res = rezistentan, jer ga proteinaze ne mogu potpuno razgraditi

PrP^{c}



Solubilni protein (42% α-uzvojnice;3% β-nabrane ploče), sastavni dio **površine stanica** (glija, neuroni, B limfociti, keratinociti i dr.), nepoznate funkcije, **nezarazan**

PrP^{sc}

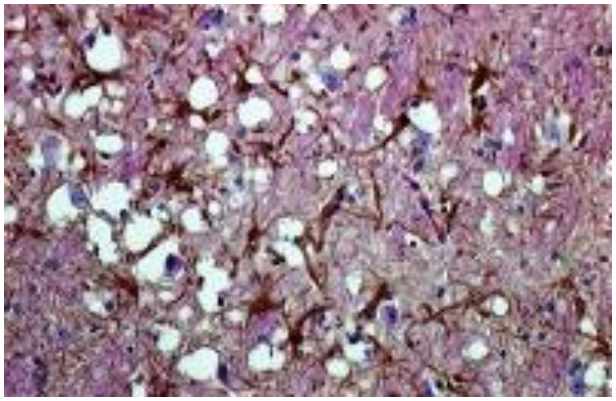


Patološka izoforma prionskog proteina (30% α-uzvojnice; 43% β-nabrane ploče), **globularan, nesolubilan**, smješten **u citoplazmatskim vezikulama, zarazan**

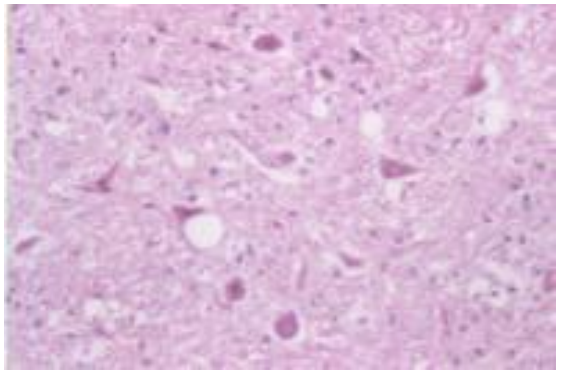
Prionske bolesti = prenosive spongioformne encefalopatije

- Prionske bolesti životinja
- Prionske bolesti čovjeka

Scrapie

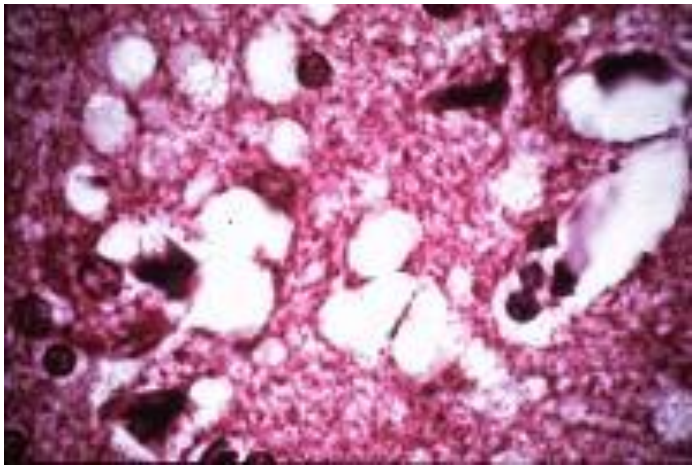


BSE

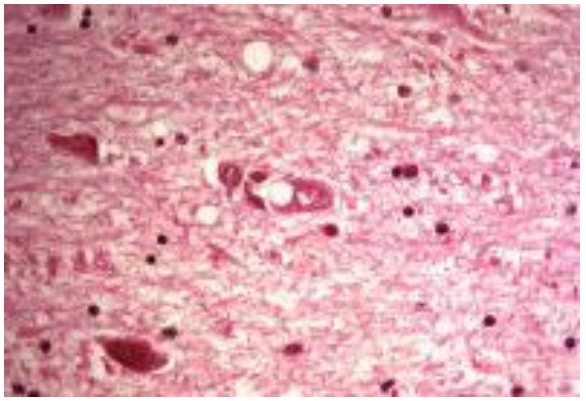


Spongioformne encefalopatije

Kuru



vCJD



Prionske bolesti čovjeka

- Spontana pojava
- Mutacije u genu za prion
- Jatrogeni prijenos
- Horizontalni prijenos (ingestija, transfuzija)

Aktuelna dijagnostika prionskih bolesti

- Dijagnoza se postavlja ***post mortem***, iznimno nakon biopsije
- Definitivna dijagnoza se osniva na neuropatološkom nalazu i identifikaciji PrP^{sc} u postmortalnom uzorku (imunohistokemija, imunoblot, ELISA)
- Plazminogen se veže na PrP^{sc}

Characteristics

- PrPc (**c**ellular, sensitive)
- Kromosom 20, kodon 129 ljudskog genoma
- molekularne težine 33-35kD
- solubilan, osjetljiv na proteinazu
- nalazi se na površini glija-stanica, neuorona CNS-a, keratinocita i brojnih drugih stanica u tijelu
- funkcija mu nije potpuno razjašnjena