



# BSE and strategies for testing

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# Outline of Seminar

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- 1. Introduction to Prion Diseases**
- 2. BSE in Cattle**
- 3. BSE Surveillance in the U.S**

# Transmissible Spongiform Encephalopathies (TSEs) or Prion Diseases

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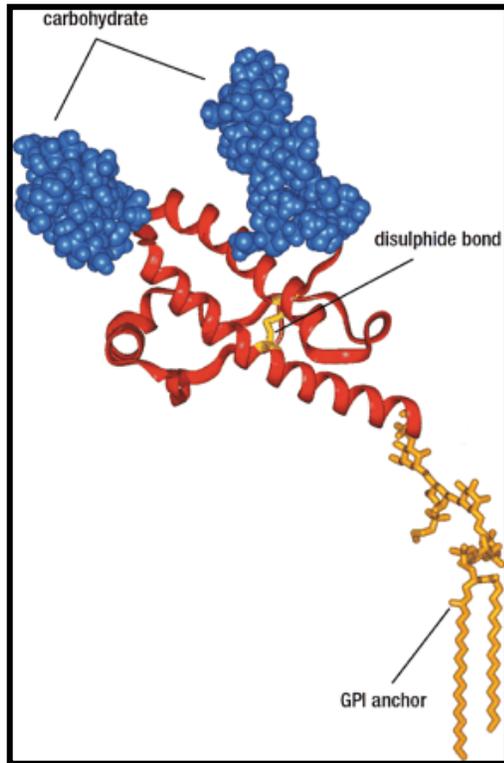
- slow, fatal, transmissible CNS diseases
- occur in variety of mammals including humans
- can be experimentally transmitted to rodents
- incubation period: months to decades (Kuru  $\leq$  40y)
- infection can occur from ingestion or parenteral inoculation
- TSEs are always fatal:
  - no effective pre- or post-clinical treatment
  - no sensitive, pre-clinical diagnostic test available
- no immune response to the prion agent

# Features of prion diseases, cont.

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- Etiology: Infectious, Sporadic, Genetic  
ALL forms **INFECTIOUS** upon subsequent passage.
- Neurodegenerative Diseases
- Short clinical course: progressive & fatal.
- Neurological impairment: cognitive, motor, sensory.
- Exact nature of infectious agent still unclear (infectious protein, prion protein: PrP<sup>Sc</sup>?)

# Physical and Biochemical Properties of PrP



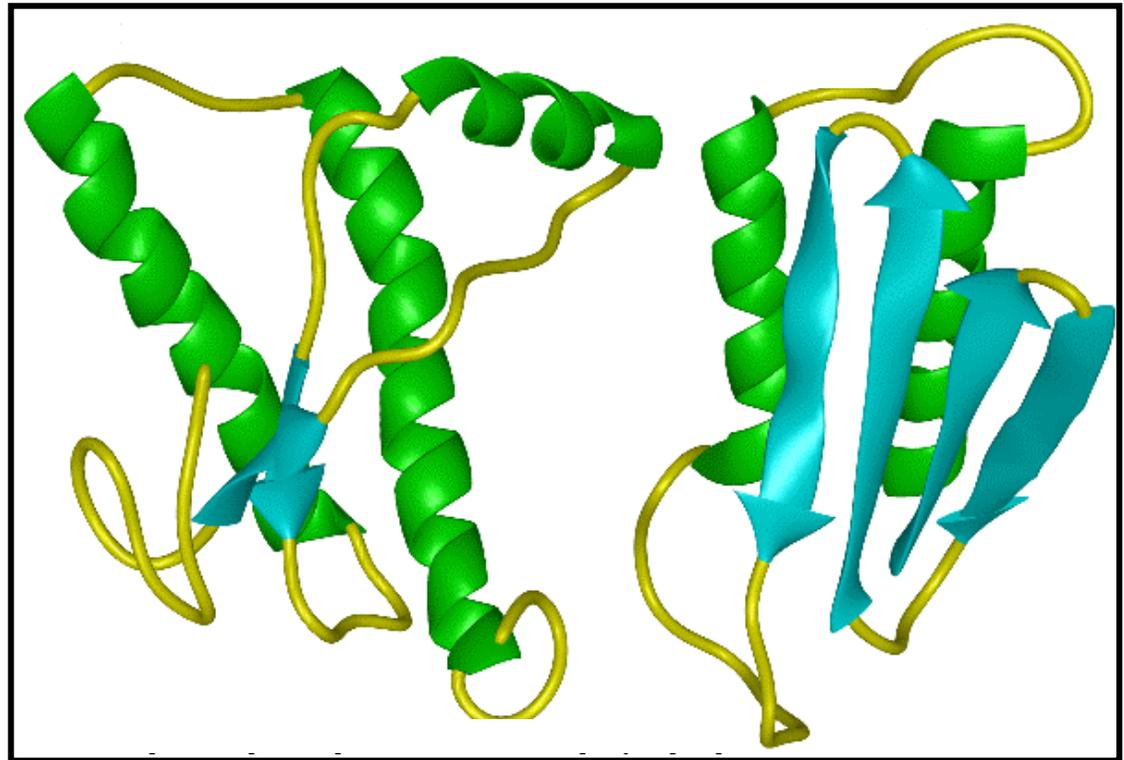
## Prion protein

33-35 kDa

Two N-linked glycosylation sites

Single disulfide bond

GPI anchor



## Normal isoform, PrP<sup>C</sup>

Proteinase K sensitive

high  $\alpha$ -helical content

soluble

globular

## Abnormal isoform, PrP<sup>Sc/res/d</sup>

Proteinase K resistant

high  $\beta$ -sheet content

insoluble

fibrillar

# Role of the Prion Protein (PrP) in Prion Diseases

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- **Normal PrP (PrP<sup>c</sup>):**

- required for infection and disease (PrP<sup>-/-</sup> mice)
- mutations in PrP<sup>c</sup> can strongly influence susceptibility to TSE disease (or even be the basis for it!)

- **Abnormal PrP (PrP<sup>sc</sup>):**

- associated with neurotoxic events in the CNS
- always associated with infectious agent

# **BSE in Cattle**

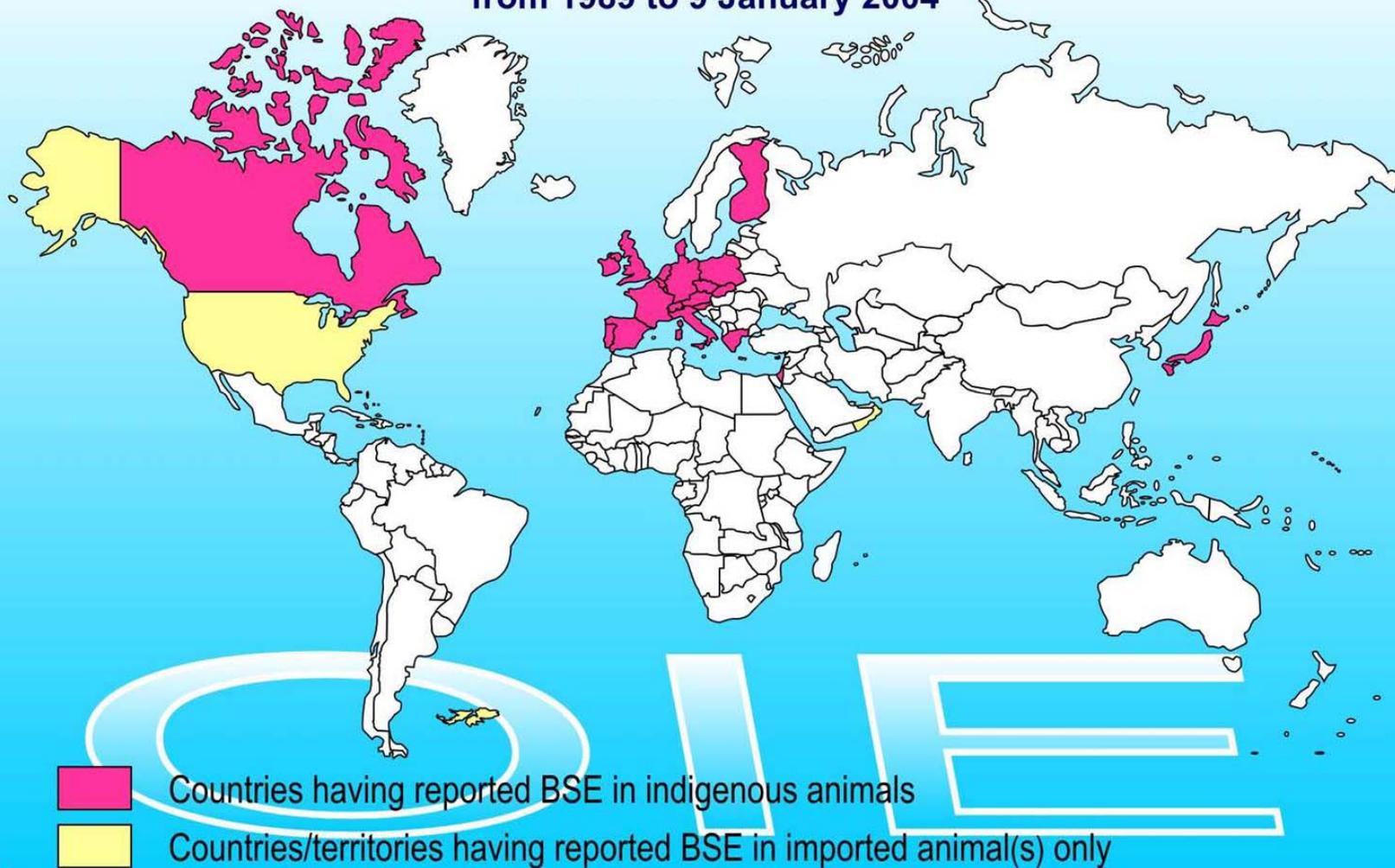
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# EPIDEMIOLOGY

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- **1986:** First case described in United Kingdom (to date more than 180,000 cases)
- Adult cattle: mean age of onset ~ 5 years
- Incubation time after oral infection: ~ 3 years, up to 8 years (depending on dose)

**Geographical Distribution of Countries that Reported at least one BSE Confirmed Case from 1989 to 9 January 2004**



# HOST RANGE

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- Cattle (*Bovidae*) incl. exotic ungulates (EUE)
- *Felidae* family (Feline Spongiform Encephalopathy - FSE)
- Humans (Variant Creutzfeldt-Jakob Disease, vCJD): consumption of BSE contaminated products
- Experimentally: sheep, goats, pigs, mice, mink and marmosets/macques

# Cattle: Infectious tissues

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Infectivity found in following tissues:

- Brain
- Spinal cord
- Trigeminal and dorsal root ganglia
- Ileum
- Tonsils (1/5)
- Retina
- Bone marrow
- **NOT** in muscle or blood!

# TRANSMISSION

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- **Ingestion** of contaminated feed (meat & bone meal, MBM)
- **No** horizontal transmission
- **Maternal** transmission questionable

# Transmission rate of BSE in cattle, primates and mice

**BSE bovine brain inoculum (dose)**

	100 g	10 g	5 g	1 g	100 mg	10 mg	1 mg	0.1 mg
<b>Cattle (oral)</b>	10/10 (100%)	7/9 (78%)	ND	7/10 (70%)	3/15 (20%)	1/15 (7%)	1/15 (7%)	
<b>RIII Mice (ic +ip)</b>						17/18 (94%)	15/17 (88%)	1/14 (7%)
<b>Primate (oral)</b>			1/2 (50%)					

**ID<sub>50</sub> primate**
**ID<sub>50</sub> cattle**
**ID<sub>50</sub> mice**

Adapted from Lasmézas et al., 2005

# BSE - typical/atypical strains?

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- Until about 2 years ago researchers assumed that only one strain of BSE exists ("typical" BSE).
- Recently "atypical" forms of BSE have been reported in France, Italy, Japan and Belgium.

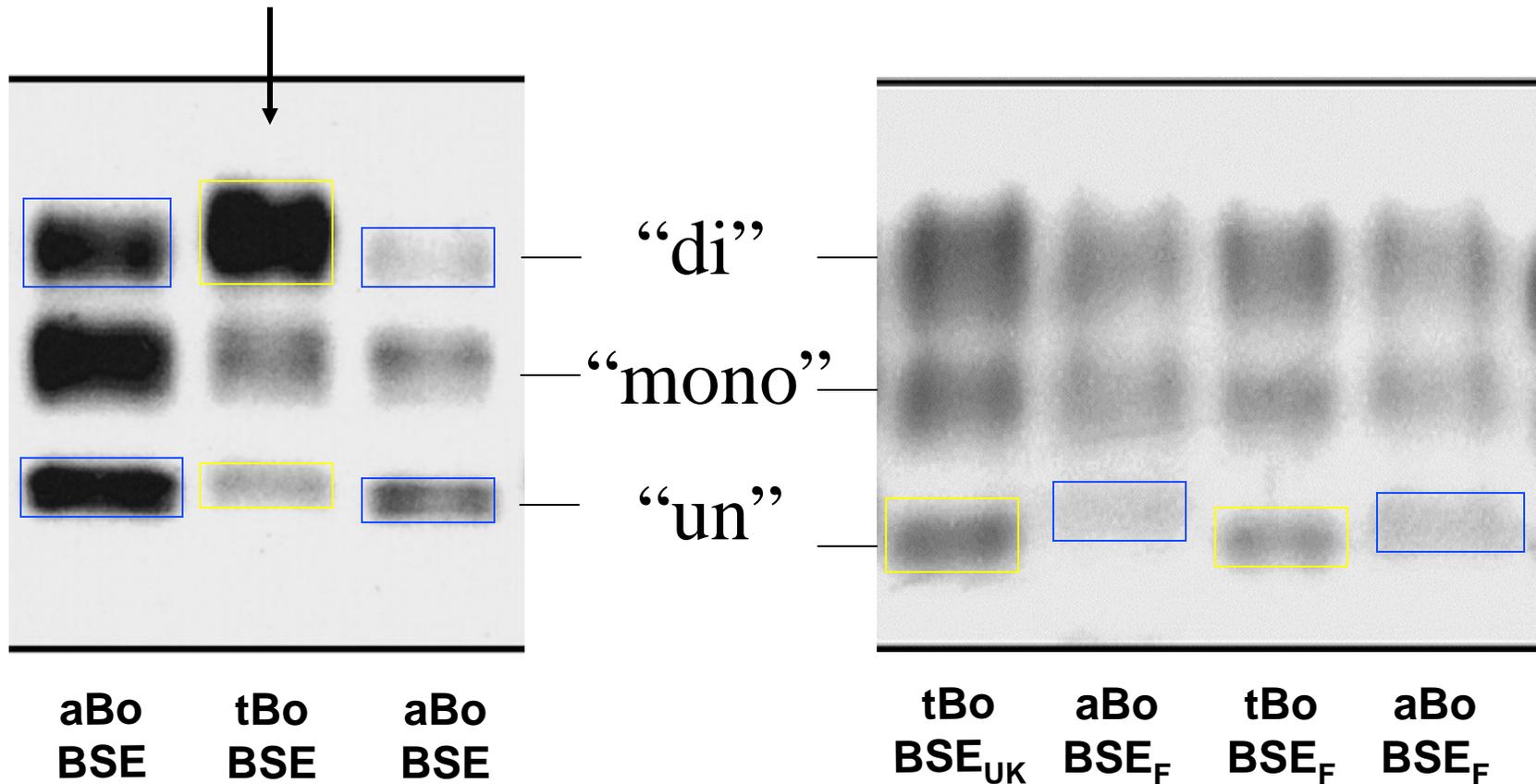
# Atypical vs. Typical BSE

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- **Spongiform changes:** different distribution; no histopathology
- **IHC:** different staining and distribution pattern; negative by IHC
- **Western Blot:** higher or lower molecular weight of unglycosylated form
- **Glycoform profile:** low content of diglycosylated isoform

# Atypical BSE cases

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Casalone et al., 2004

Bacarabe et al., 2003

# Atypical BSE

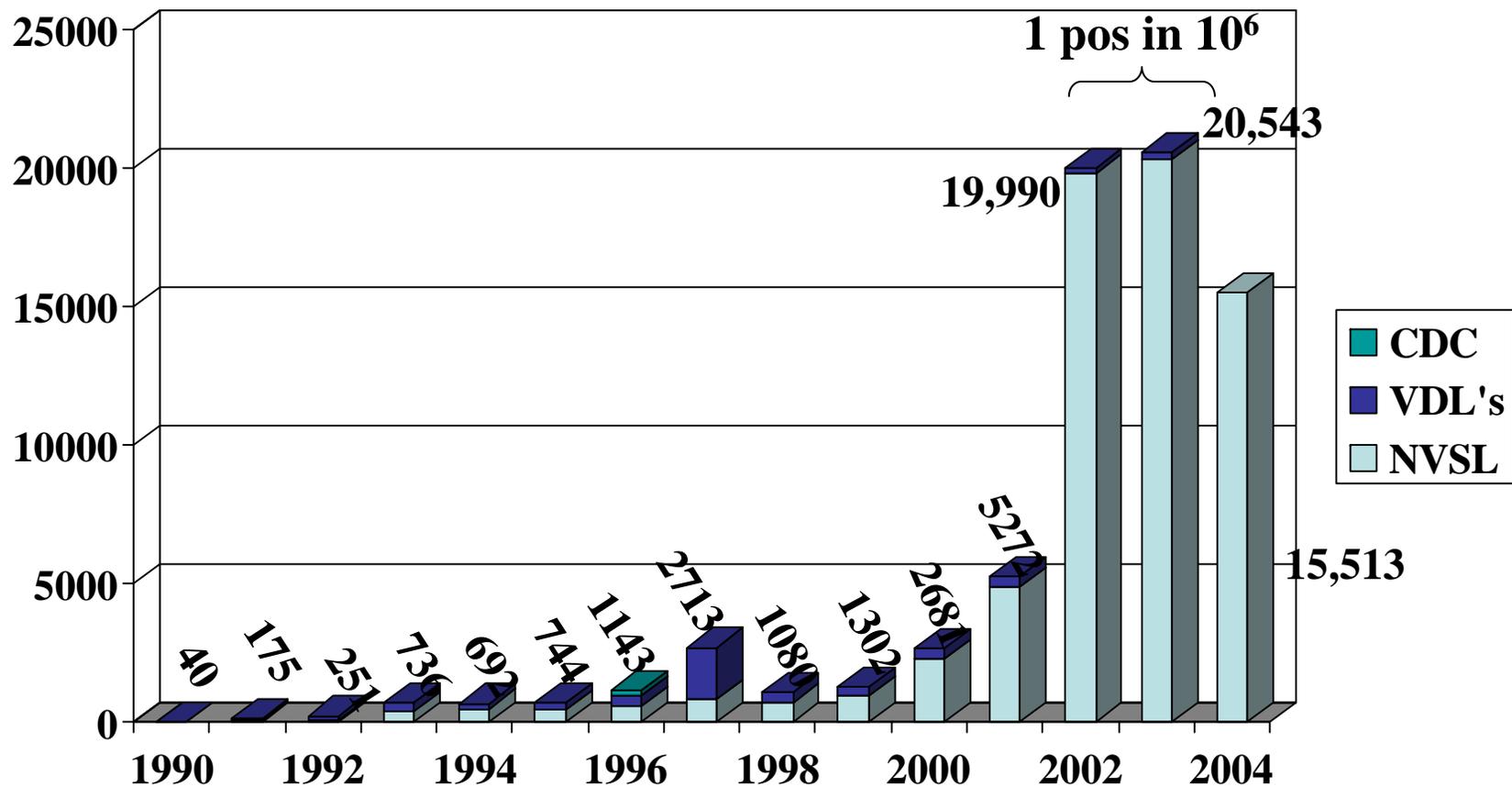
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- **Transmission attempts:** To this date no successful transmission into cattle, "bovinized" mice or other animals!
- **Question:** Is "atypical" BSE infectious?

# **BSE Surveillance in the U.S. (APHIS-VS)**

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# BSE Surveillance in the U.S. 1990 - 2004 (before June 1; IHC)



Lisa Ferguson, APHIS

# BSE Surveillance (since June 1, 2004)

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- APHIS, in cooperation with FSIS, and FDA, has implemented an **intensive national BSE surveillance plan**.
- This **one-time effort** will help to define whether BSE is actually present in the U.S. cattle population and if so, provide better estimates of the level of disease.
- The goal of this plan is to test as many adult cattle in the **targeted high-risk population** as possible in a 12-18 month period (plus 20,000 healthy slaughter).
- Animal health purpose- **NOT food safety!**

# BSE Surveillance- cont.

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- If a total of **201,000 samples** is collected, this level of sampling would allow us to detect BSE at the rate of 1 positive in 10 million adult cattle at a **95% confidence level**.
- If a total of at least **268,500 samples** is collected, this level of sampling would allow us to detect BSE at the same rate at a **99% confidence level**.

Enhanced program could detect BSE if there were **five positive animals** in the targeted population in the entire U.S.

# BSE Surveillance: Targeted Cattle Population

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Age - Over 30 months as evidenced by the eruption of at least one of the second set of permanent incisors

1. Non-ambulatory cattle
2. Cattle with CNS signs and/or rabies negative
3. Cattle exhibiting other signs that may be associated with BSE
4. Dead cattle

# Rationale for surveillance targeted at high risk cattle (EU experience)

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## BSE surveillance in France (2001):

1. ~1/5 **clinical suspects** positive for BSE
2. ~1/1,300 **non-ambulatory** cattle positive for BSE
3. ~1/30,000 **healthy slaughter** positive for BSE

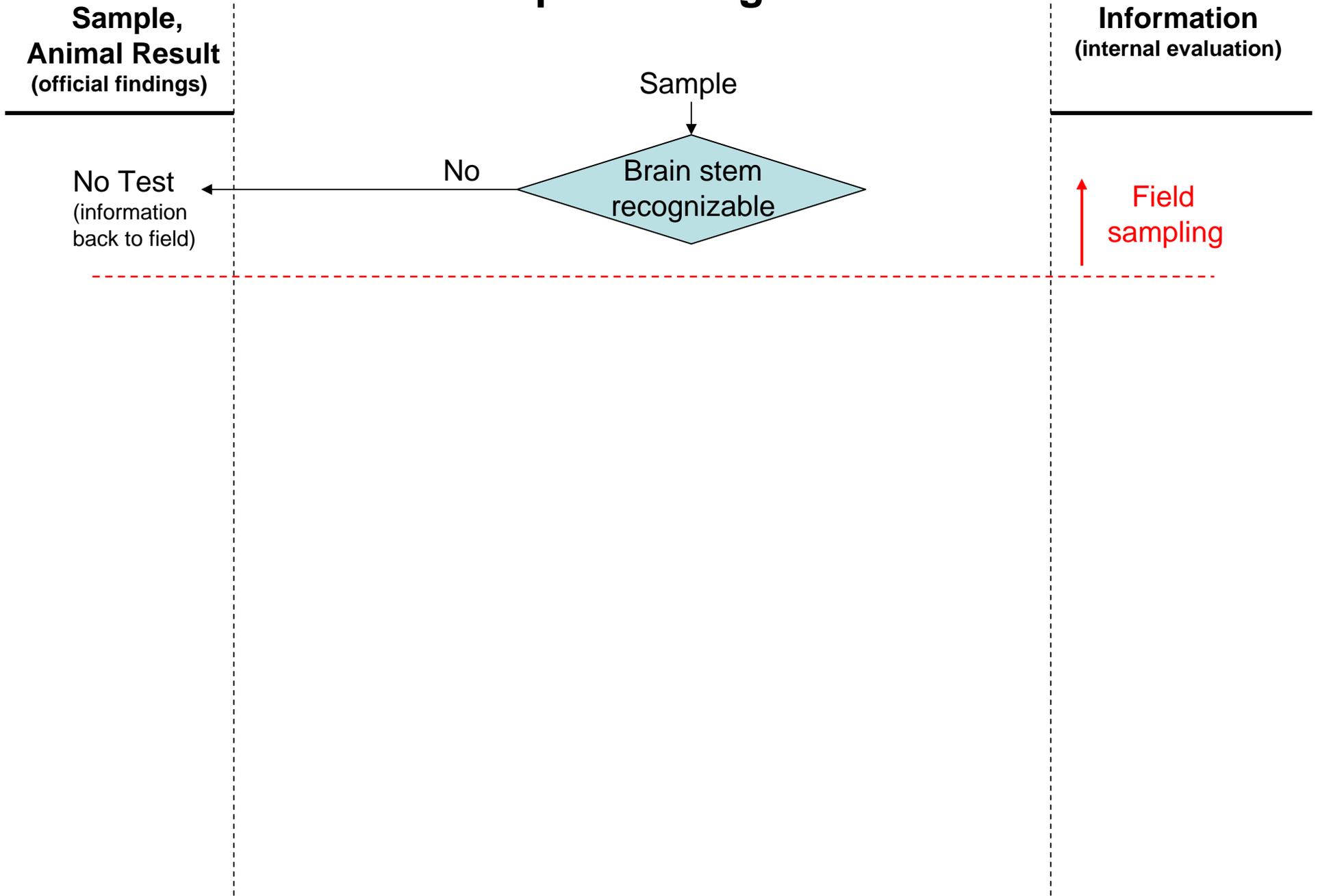
**Targeted surveillance more effective than random sampling in detecting BSE-infected animals**

# BSE Sampling & Testing

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- Sampling at farm, slaughter & rendering facilities, veterinary clinics, livestock auctions & public health laboratories.
- Testing conducted at NVSL and 7 geographically placed contract laboratories (SVDLs)

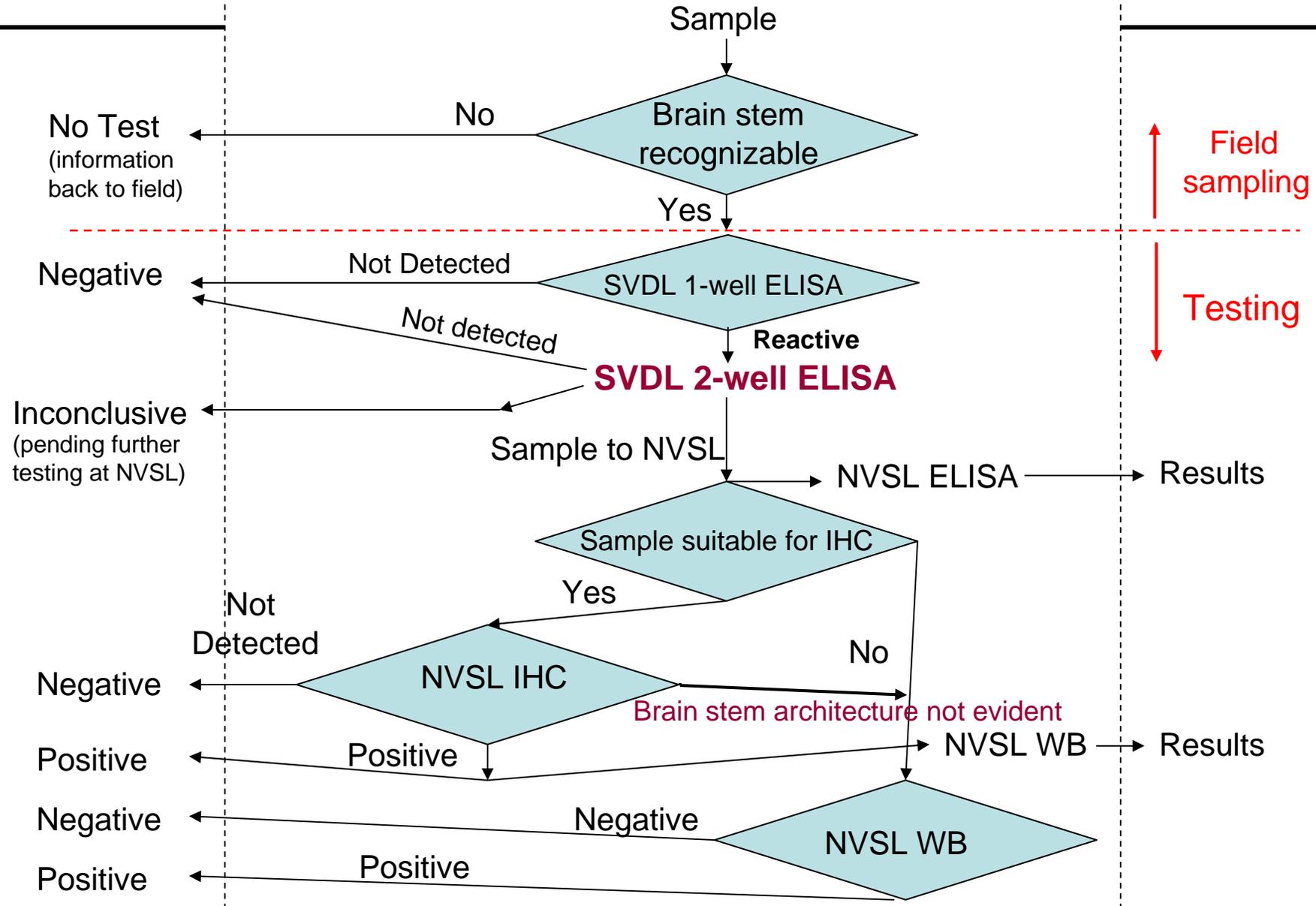
# Sample/Testing Flow



# Sample/Testing Flow

**Sample,  
Animal Result**  
(official findings)

**Information**  
(internal evaluation)



# Laboratory Diagnosis of BSE

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**A laboratory diagnosis/case definition of BSE in the United States will be made if one of the following criteria is fulfilled:**

- 1. Positive results by Rapid test and IHC.**
- 2. Positive results by Rapid test and Western Blot - in case sample is not suitable for IHC or brain stem architecture is not evident.**
- 3. Positive results by IHC only - in case no appropriate fresh brain tissue ("formalin fixed") is available to employ either a Rapid or Western Blot test.**

# Characteristics of BSE situation in the U.S.

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**Yield of positive cases from surveillance of high risk cattle:**

- 1. One (imported) case from ~ 70,000 samples up to May 2004**
- 2. No additional cases from >240,00,000 high risk cattle tested by Rapid test since June 1, 2004**

**BSE - if present – is a rare disease in the U.S.**