U.S. Department of Agriculture

Agricultural Outlook Forum 2005

February 25, 2005

BSE AND STRATEGIES FOR TESTING

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BSE and strategies for testing

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Outline of Seminar

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

Transmissible Spongiform Encephalopathies (TSEs) or Prion Diseases

- 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.

- Etiology: Infectious, Sporadic, Genetic

  ALL forms INFECTIOUS upon subsequent passage.

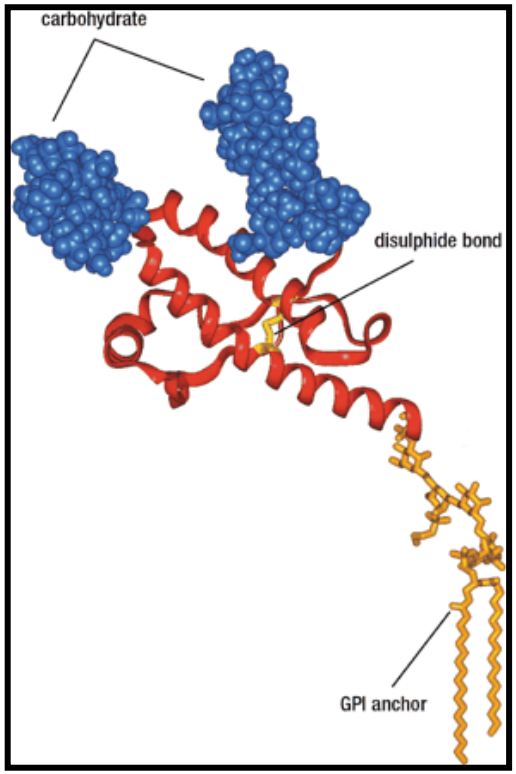
- Neurodegenerative Diseases

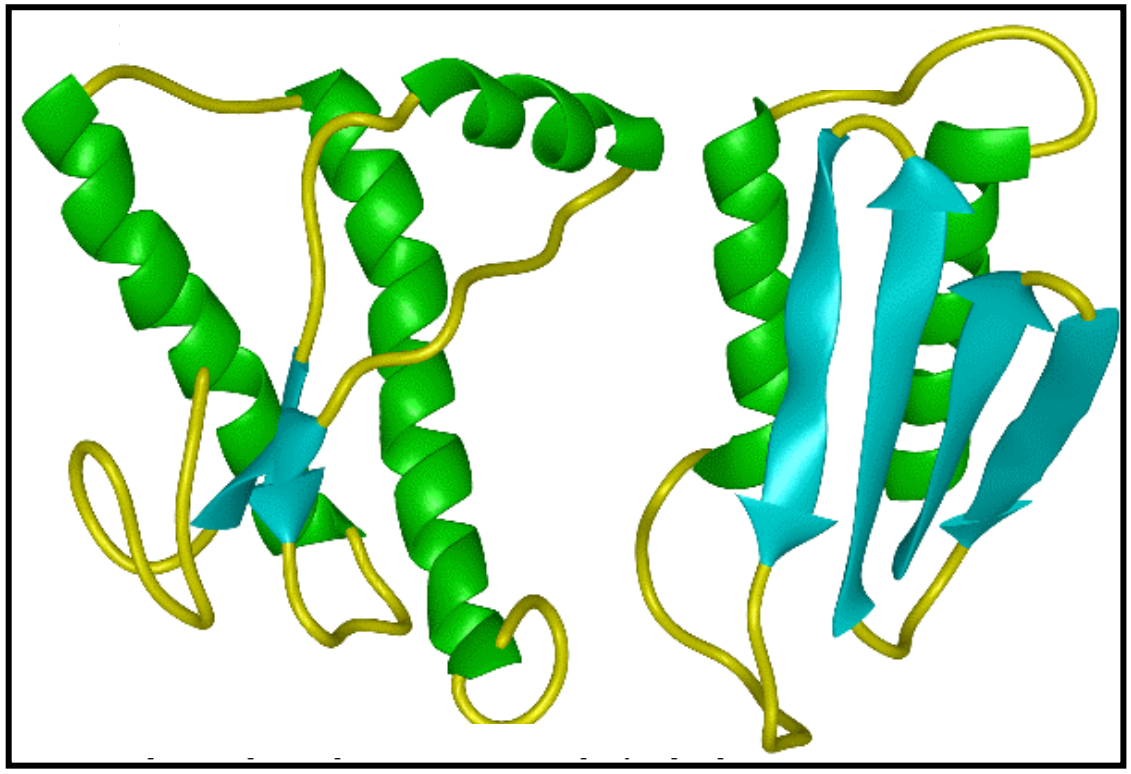
- Short clinical course: progressive & fatal.

- Neurological impairment: cognitive, motor, sensory.

- Exact nature of infectious agent still unclear (infectious protein, prion protein: PrP^{Sc}?)

Physical and Biochemical Properties of PrP





Prion protein

33-35 kDa

Two N-linked glycosylation sites

Single disulfide bond

GPI anchor

Normal isoform, PrP^{C}	**Abnormal isoform, $PrP^{Sc/res/d}$**
Proteinase K sensitive	**Proteinase K resistant**
high α-helical content	**high β-sheet content**
soluble	**insoluble**
globular	**fibrillar**

Prusiner, 2001

Conformational Isomers

Role of the Prion Protein (PrP) in Prion Diseases

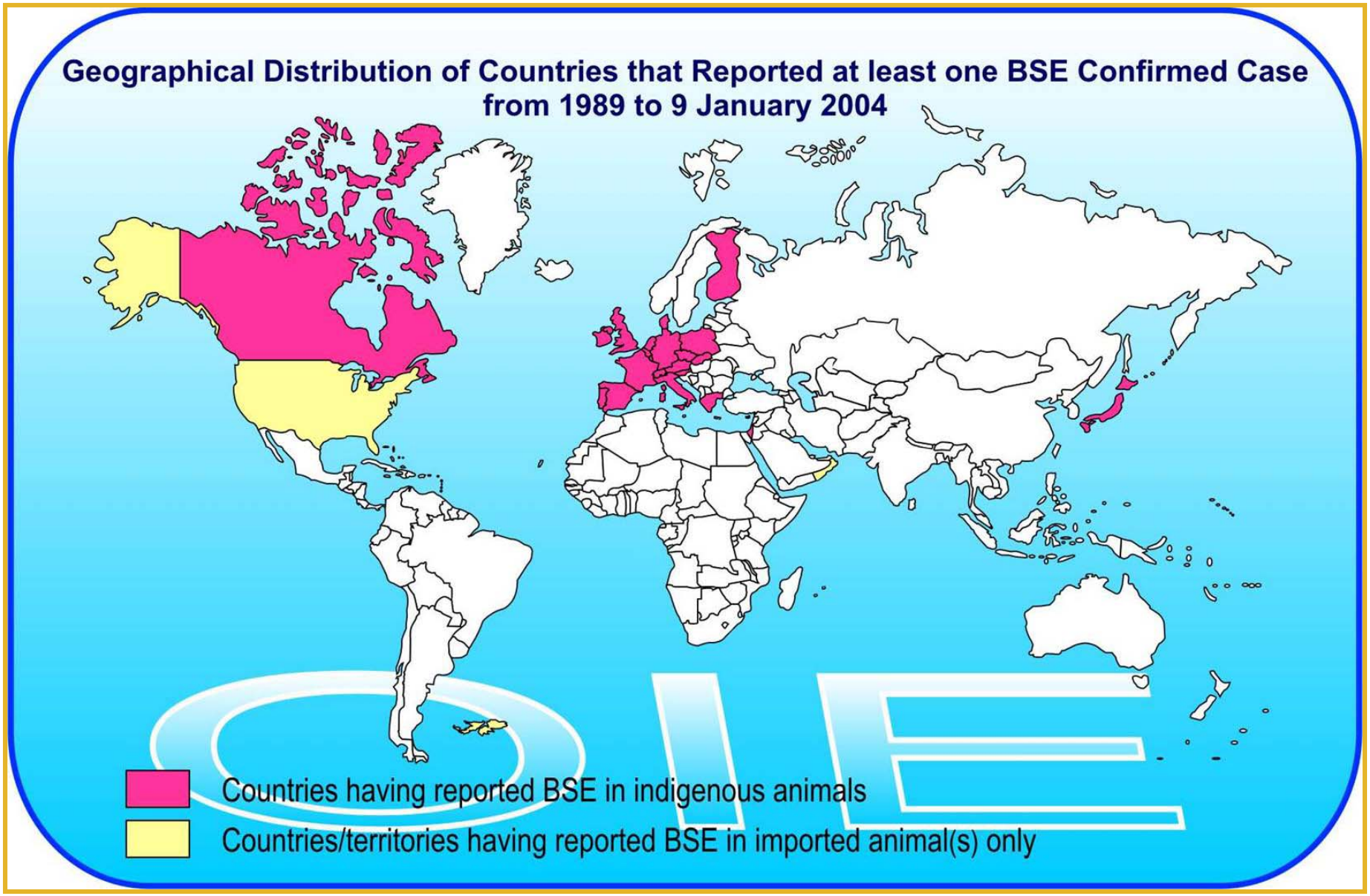
- **Normal PrP (PrP^{c}):**
  - **required for infection and disease ($PrP^{-/-}$ mice)**
  - **mutations in PrP^{c} can strongly influence susceptibility to TSE disease (or even be the basis for it!)**

- **Abnormal PrP (PrP^{sc}):**
  - **associated with neurotoxic events in the CNS**
  - **always associated with infectious agent**

BSE in Cattle

EPIDEMIOLOGY

- **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)



http://www.oie.int/Cartes/BSE/a_Monde_BSE.htm

HOST RANGE

- 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/macaques

Cattle: Infectious tissues

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

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

Transmission rate of BSE in cattle, primates and mice

ID_{50} primate

ID_{50} cattle

ID_{50} mice

BSE bovine brain inoculum (dose)

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

Adapted from Lasmézas et al., 2005

BSE – typical/atypical strains?

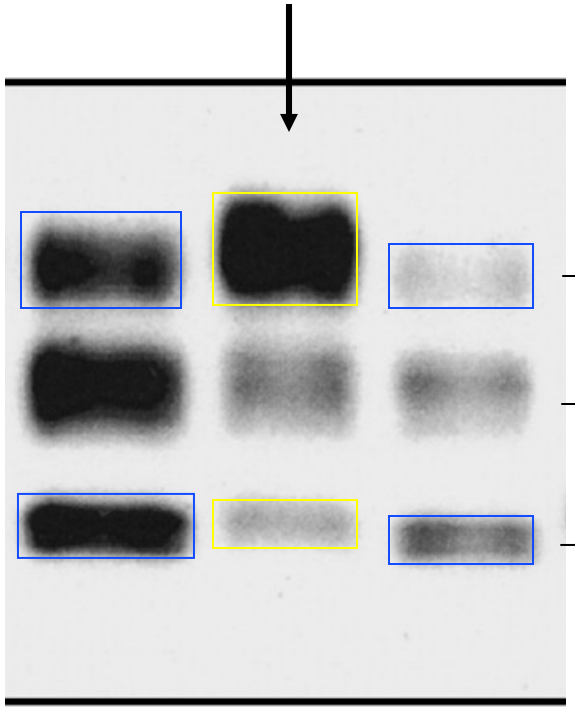
- Until about 2 years ago researchers assumed that only one strain of BSE exists ("typical" BSE).

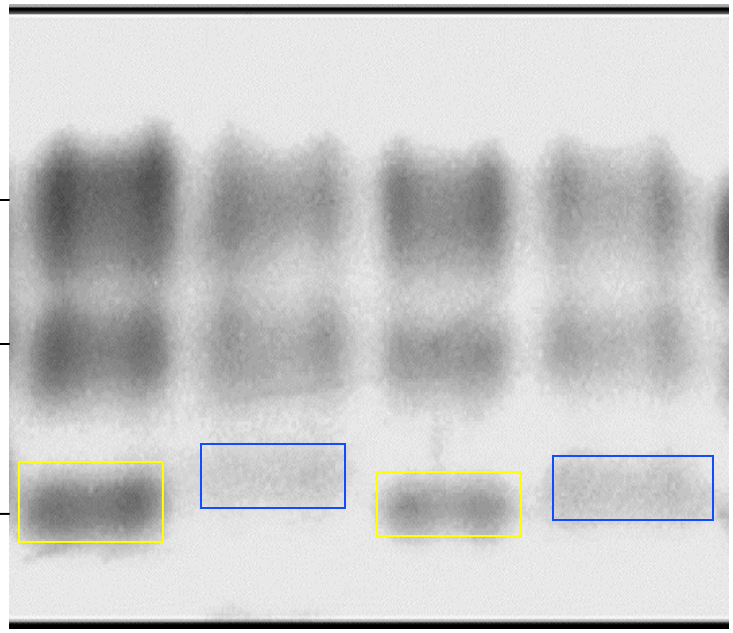
- Recently "atypical" forms of BSE have been reported in France, Italy, Japan and Belgium.

Atypical vs. Typical BSE

- **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





"di"

"mono"

"un"

aBo BSE | tBo BSE | aBo BSE

tBo BSE_{UK} | aBo BSE_F | tBo BSE_F | aBo BSE_F

Casalone et al., 2004

Bacarabe et al., 2003

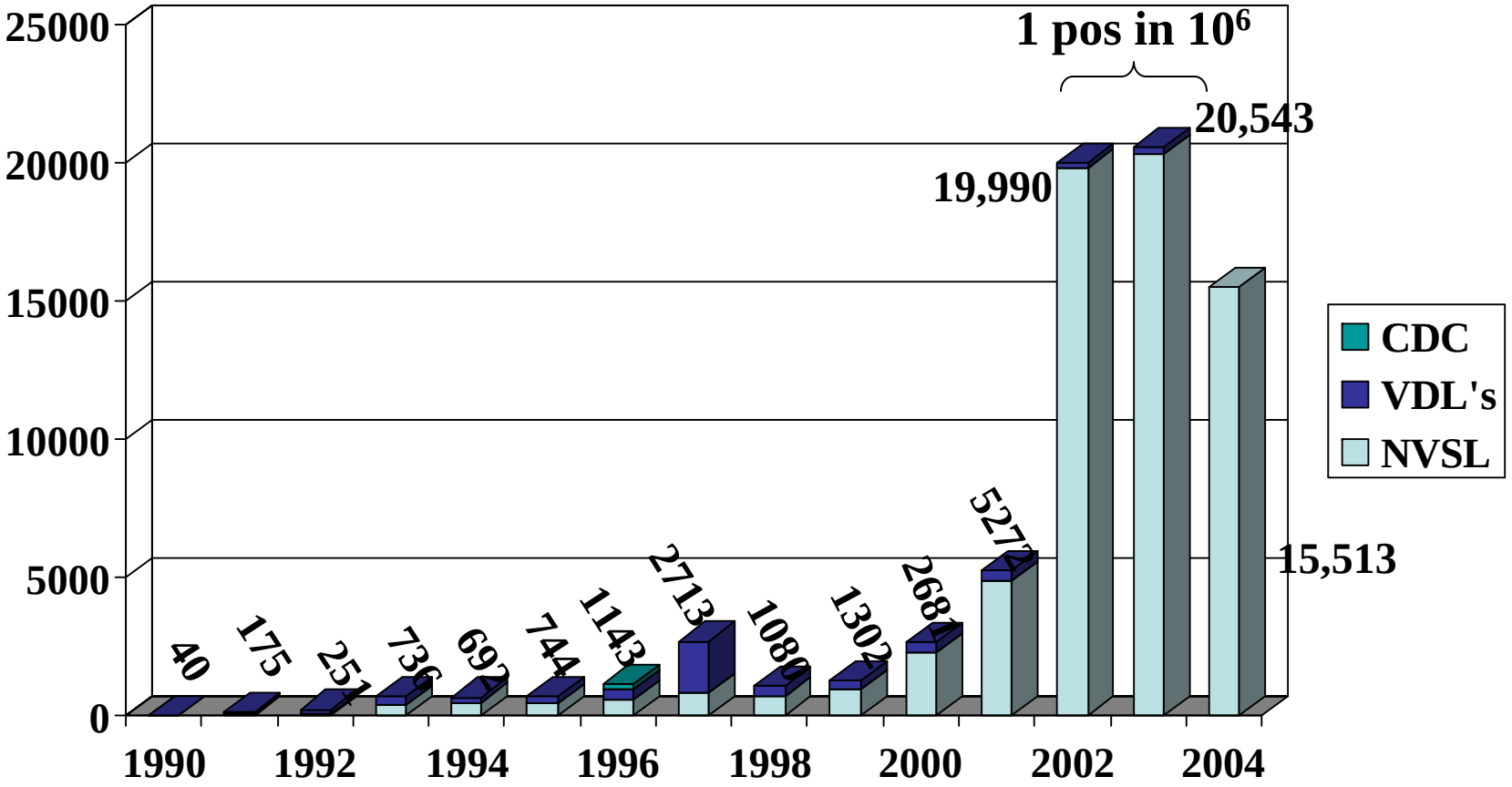
Atypical BSE

- **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)

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



Lisa Ferguson, APHIS

BSE Surveillance (since June 1, 2004)

- 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.

- 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

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)

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

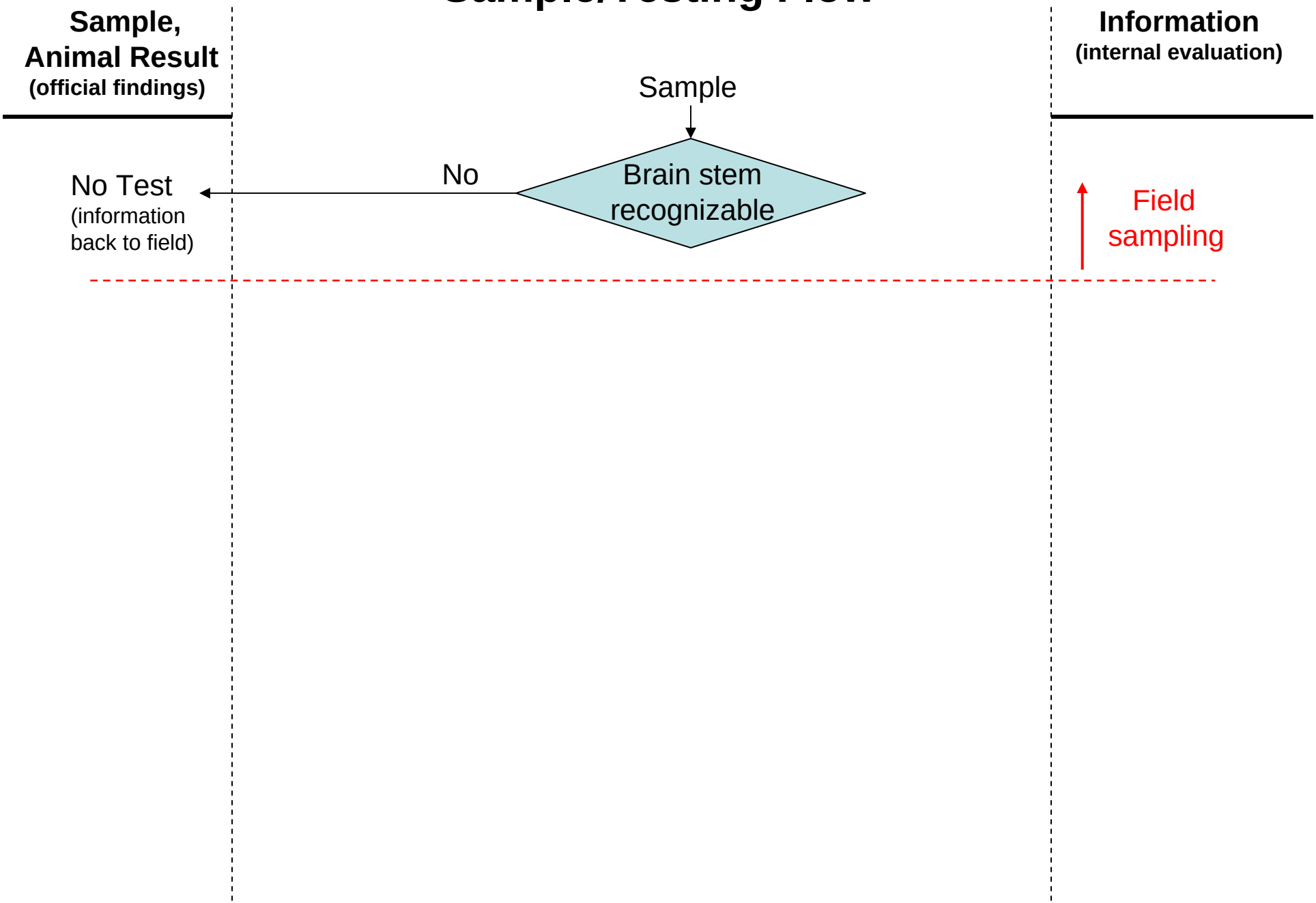
Targeted surveillance more effective than random sampling in detecting BSE-infected animals

BSE Sampling & Testing

- 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



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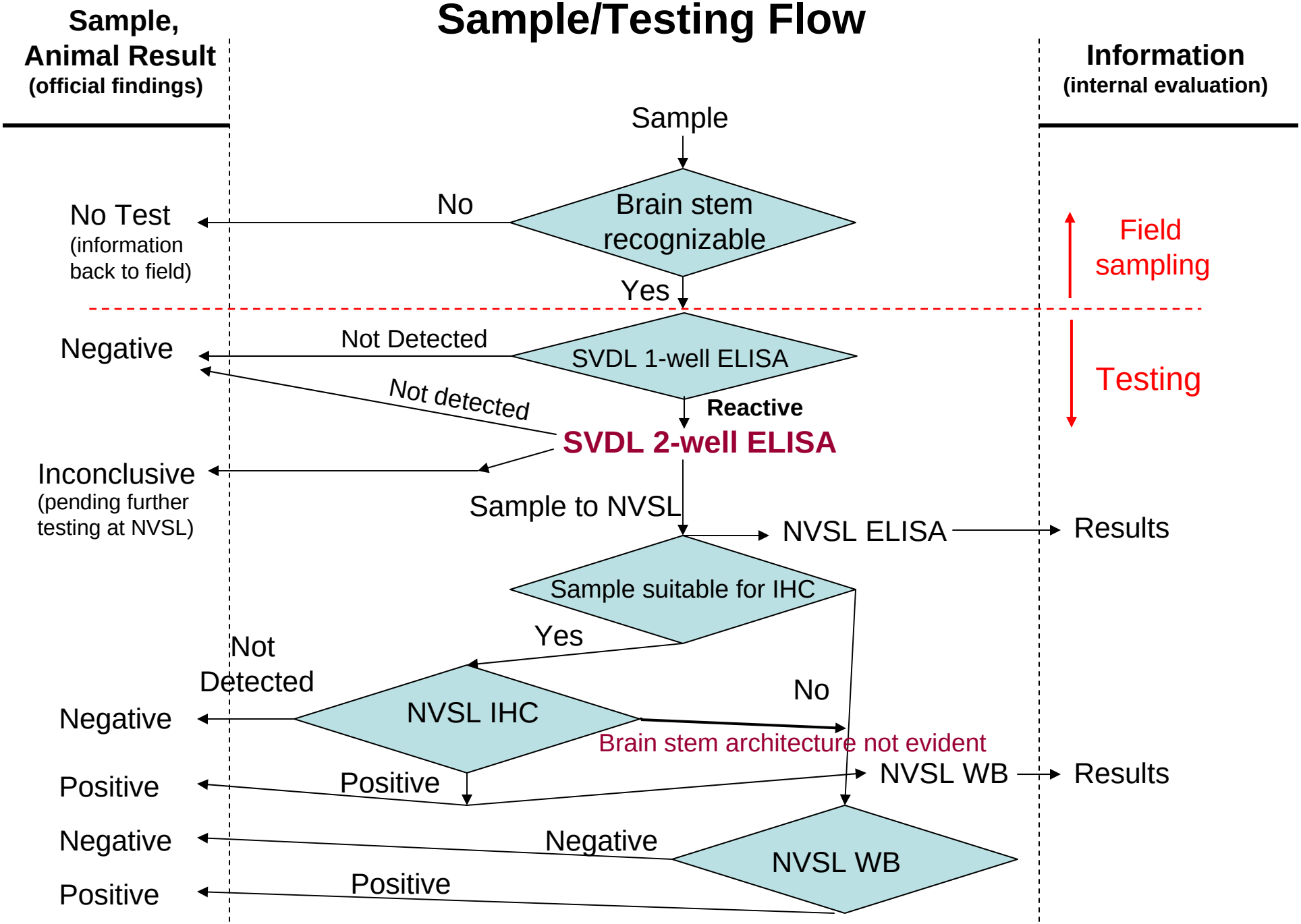

Laboratory Diagnosis of BSE

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.

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.