BSE in Canada

Dr. med.vet. Stefanie Czub, Canadian & OIE Reference Laboratories for BSE, CFIA Lethbridge/AB

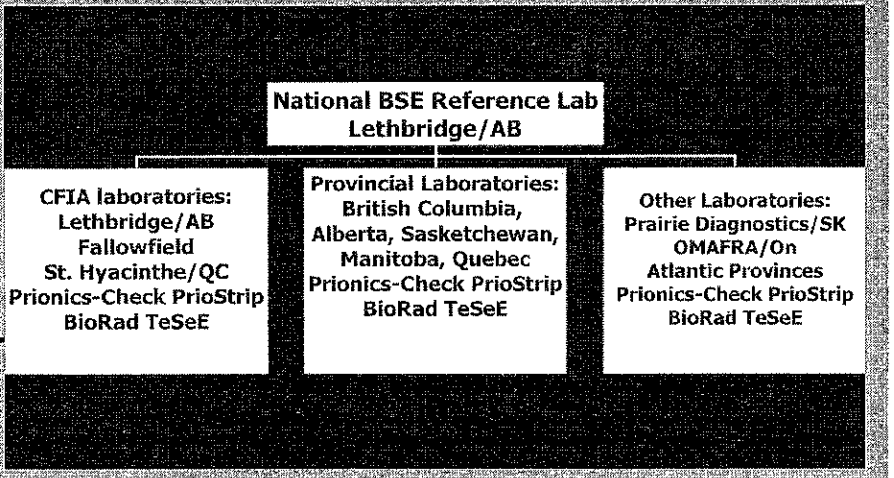
Mandate BSE reference laboratory

- Confirmation of suspect samples
- QA for BSE network laboratories (e.g. biannual proficiency panels for IHC + rapid tests)
- Validation of test methods (rapid tests, IHC)
- BSE surveillance
- Applied research (APRI, HC, PHAC, Friedrich-Loeffler-Insitute, NIAH/Japan)
- Training (pathologists, laboratory scientists, technologists, technicians)
- Teaching (in-house, outside CFIA)
- Participation in German biannual "ring trial"
- Accreditation: ISO 17025, AAVLD IHC

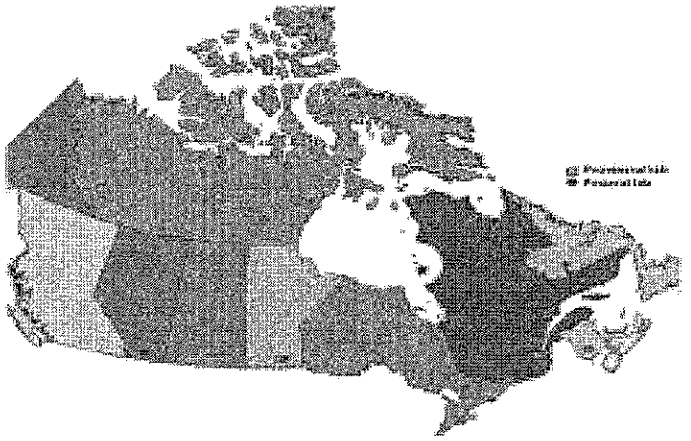
Canadian BSE Safeguards

- 1990: Federally reportable disease
- 1991: National BSE surveillance program
- 1997: Ruminant to ruminant feed ban
- 2001: National TSE network , Canadian Cattle Identification Program
- 1993: BSE in an UK import (from 1987)
- May 2003: 1st indigenous BSE case. Total number of BSE cases: 18 (September 2012). 2/18 atypical BSE
- > May 2003: category III country classification (minimum risk)
- May 2007: controlled risk category (OIE)
- June 2007: enhanced feed & SRM ban

Canadian BSE Network



Canadian TSE network



BSE Surveillance:

1. OTM cattle displaying behavioural or clinical signs consistent with BSE (highest likelihood)
2. OTM cattle that are non-ambulatory, emergency slaughtered, or condemned ante-mortem (2nd highest)
3. OTM cattle found dead on farm, during transport or at abattoir (3rd highest)

2005 OIE Terrestrial Animal Health Code BSE Surveillance Chapter

		AGE CATEGORIES				
	Subgroup	>/= 1 YR < 2 YR	>/=2YR < 4 YR	>/=4 YR < 7 YR	>/=7YR < 9 YR	>/=9YR
Point Allocation	CLINICAL SUSPECT[1]	N/A	260	750	220	45
	CASUALTY SLAUGHTER[2]	0.4	0.4	1.6	0.7	0.2
	FALLEN STOCK[3]	0.2	0.2	0.9	0.4	0.1
	ROUTINE SLAUGHTER[4]	0.01	0.1	0.2	0.1	0.0

1. "... cattle > 30 months of age displaying behavioural or clinical signs consistent with BSE" *(refractory to tx., progressive behavioural or neurological signs without infection)*

2. "... cattle > 30 months of age that are non-ambulatory, recumbent, unable to rise or to walk without assistance, cattle over 30 months of age sent for emergency slaughter or condemned at ante-mortem inspection (casualty or emergency slaughter, or downer cattle)" *(includes downers, diseased)*

3. "... cattle > 30 months of age which are found dead on farm, during transport or at an abattoir (fallen stock)"

4. "... cattle > 36 months of age at routine slaughter"

BSE surveillance testing

Network Laboratories perform BSE screening tests & report negative results electronically

Inconclusive samples at the network labs to BSE reference lab for confirmation

Extensive confirmatory testing on suspect samples at BSE reference lab

Initial Reactor Flowchart **BSE Reference Lab 2010 version4**

"Initial Reactor" (non-negative or abnormal reactivity) in screening test

⇩

Immediately contact BSE National Reference Lab (Dr. Stefanie Czub) (contact list attached)
Email scans and all printouts related to the screening test

⇩

Recut sample rostral of original sampling site

Repeat the original **and** re-cut homogenates in duplicate on the same run

REACTIVE

NON-REACTIVE

⇩

"Inconclusive test result" Send all homogenates and all tissues to BSE RL (including all scans and printouts)

(contact Dr. Stefanie Czub to notify of shipment)

Sample now considered a **CN sample**

(See CN flowchart)

Notify the RL: Dr. S.Czub. Send all homogenates and all tissues (including all scans and printouts) to the BSE RL as a **QA sample**

BSE confirmatory testing scheme:

- Histopathology: serial sections of obex & other brain level (if available), H&E, LFB

- Rapid tests, Prionics-Check & Hybrid WBs (C-type >atypical BSE>CWD)

- Immunohistochemistry: ~ 8 anti-PrP antibodies & GFAP, Dako Envision Plus detection system, 1 IHC Repeat

- SAF/OIE ImmunoBlot

CN Flowchart **BSE Reference Lab 2010 version 4**

REACTIVE at Network Lab

⇩

"Inconclusive test result"

Send all homogenates and all tissues to BSE RL in Lethbridge (including all scans and printouts)

(contact Dr. Stefanie Czub to notify of shipment). Now considered a **CN sample**

⇩

BSE RL – Repeat with Rapid Test

REACTIVE

NON-REACTIVE

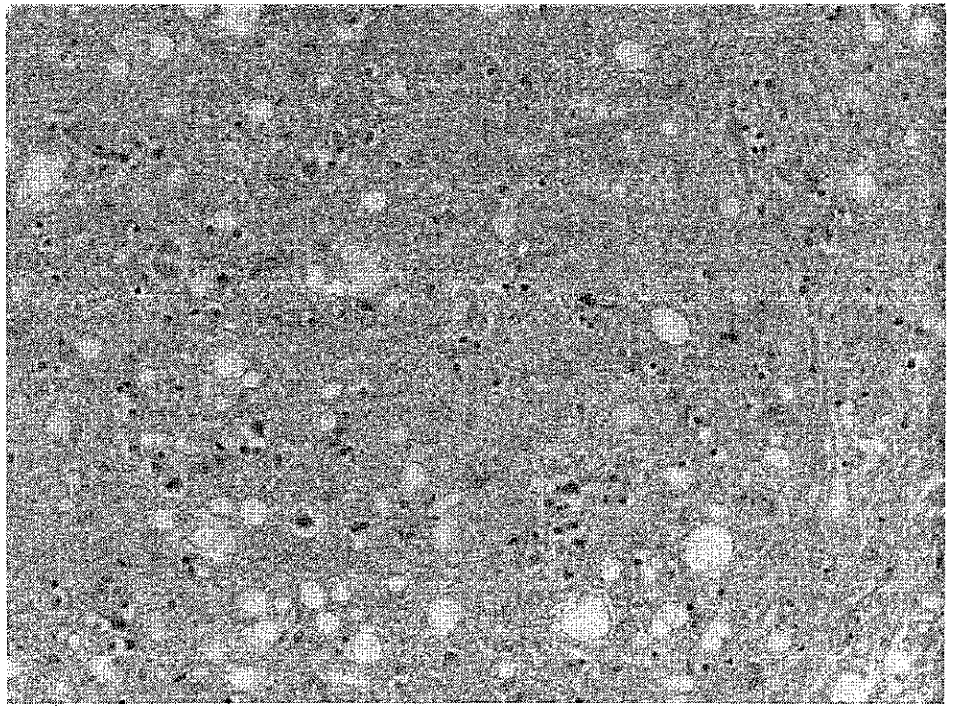
⇩

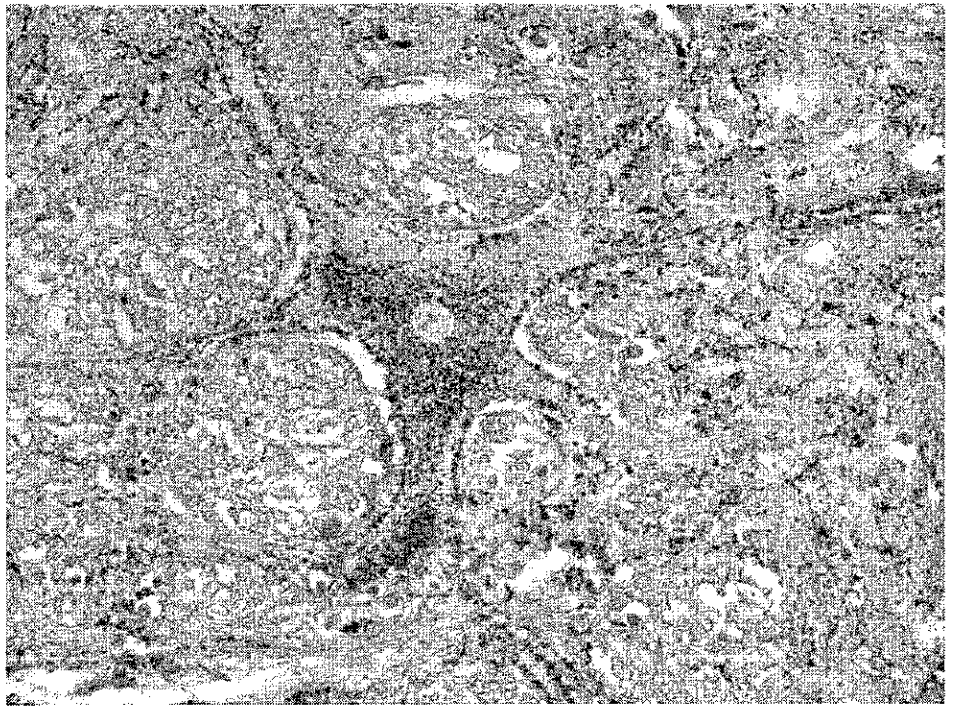
Suspect **–confirmation (CN procedure)**

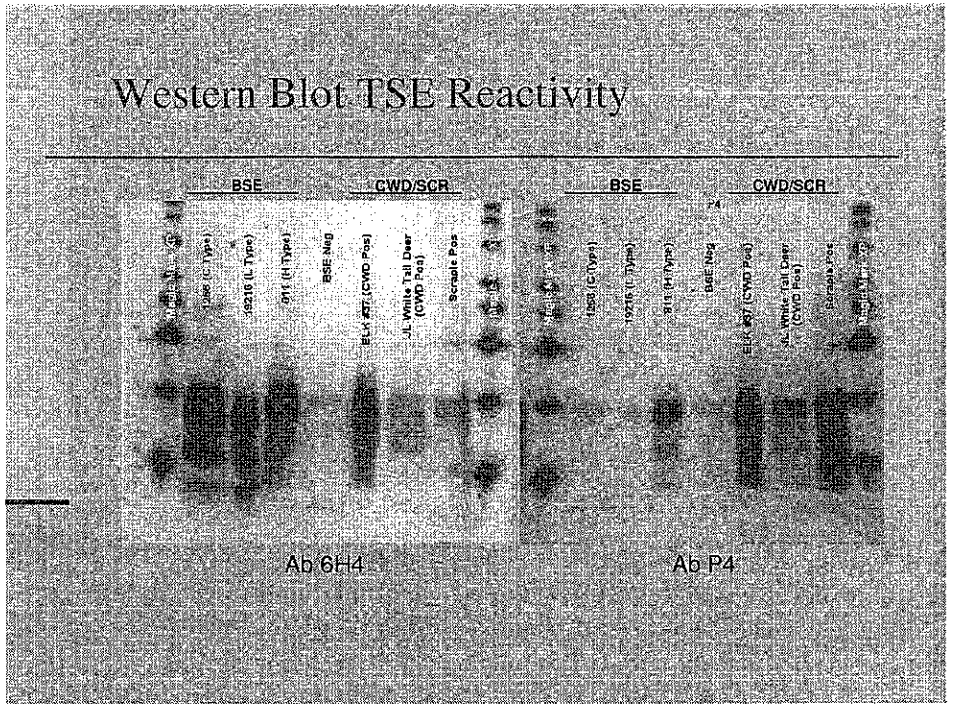
1. IHC or
2. SAF/OIE Immunoblot (poor quality samples)

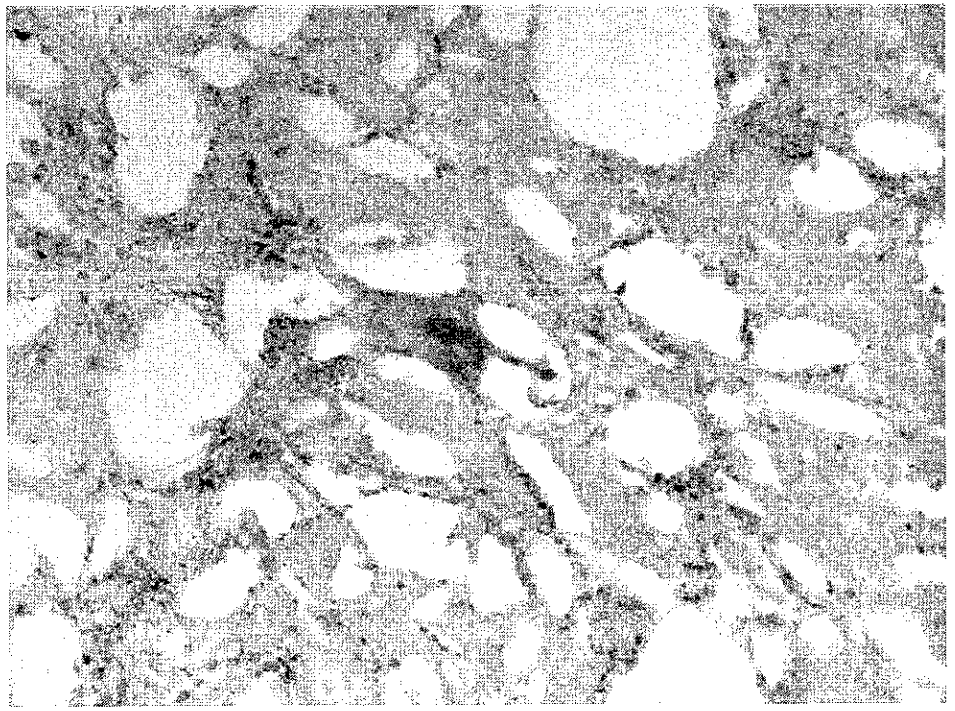
1. IHC or
2. SAF/OIE immunoblot (poor quality samples)

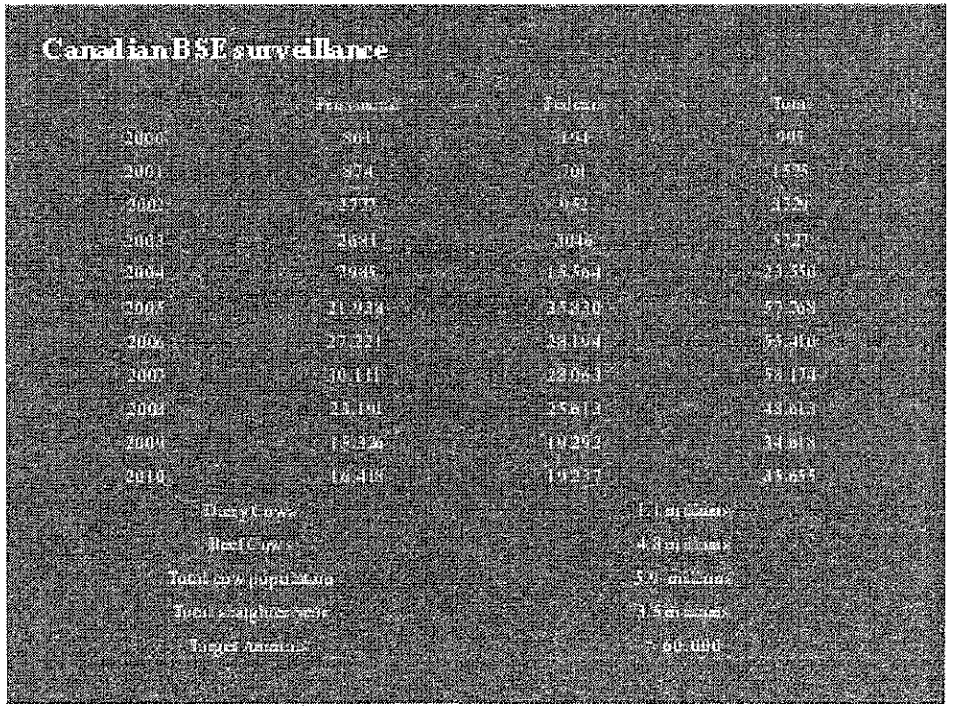
Refer to the TSE Quality Guidelines section 4.2



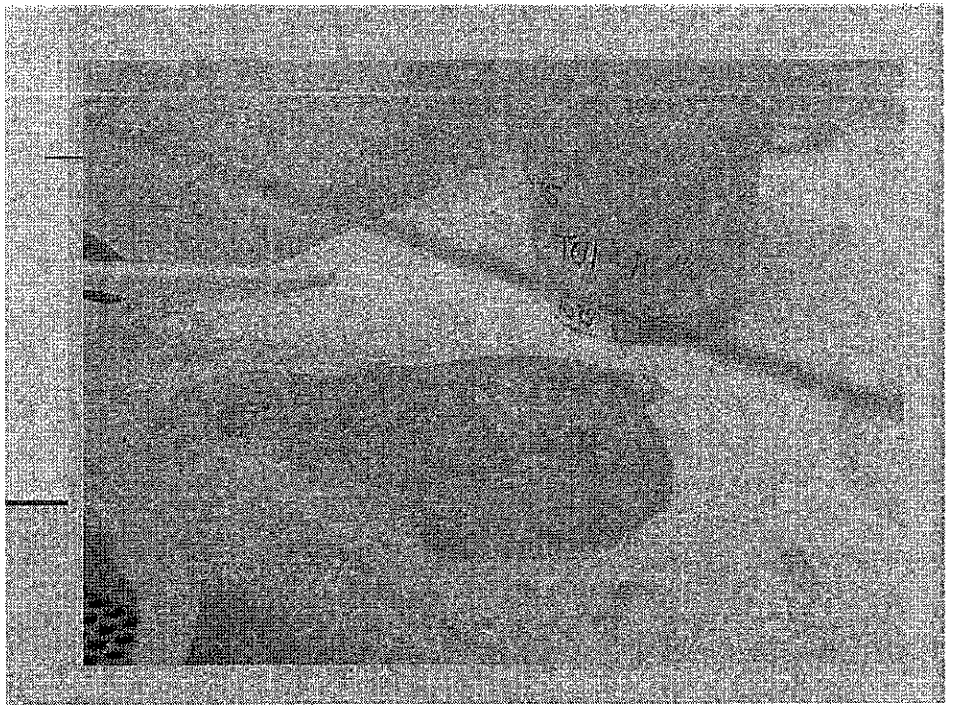



Western Blot TSE Reactivity
BSE
CWD/SCR
BSE
CWD/SCR
BSE Neg
ELK #27 (CWD Pos)
Scrapie Pos
Ab 6H4
Ab P4



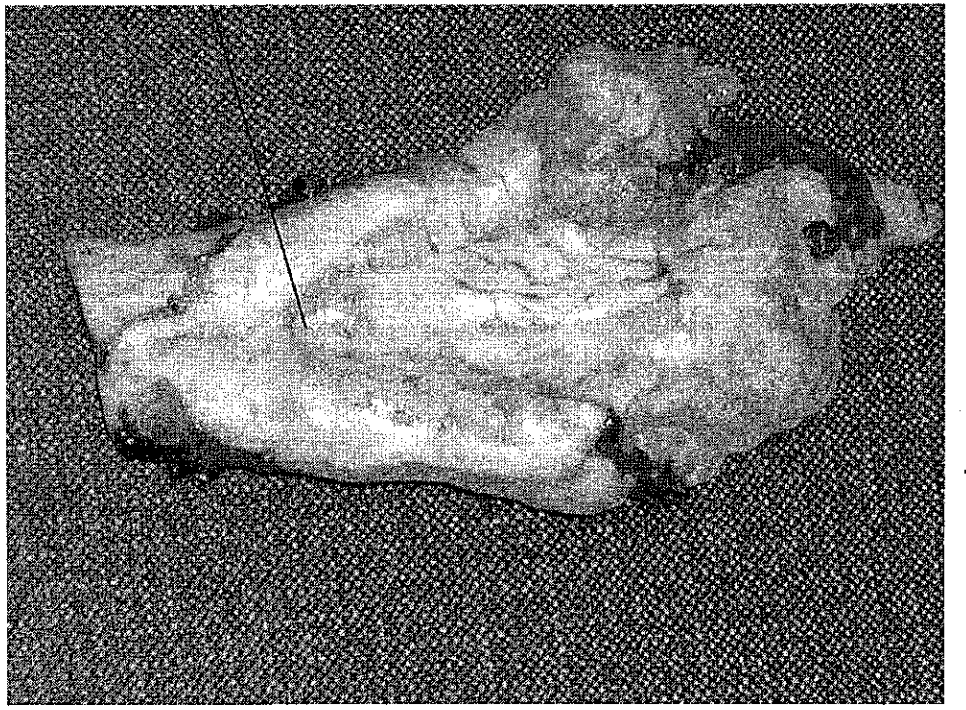

Canadian BSE surveillance

	Confirmation	Cattle Type	DOB	Age (Years)	Born
1	20/05/2003	Black Angus X	22/03/1997	5.8	SK
2	02/01/2005	Holstein	05/10/1996	8.2	AB
3	11/01/2005	Charolais	21/03/1998	6.8	AB
4	22/01/2006	Holsteinx Hereford	15/04/2000	5.8	AB
5	16/04/2006	Holsteinx Charolais	29/04/2000	5.9	BC
6	03/07/2006	Charolais X	~ 1990	~ 16	MB ; H-type BSE
7	13/07/2006	Jersey	22/04/2002	4.2	AB
8	23/08/2006	Charolais X	1996 - 1998	8 - 10	AB ?
9	07/02/2007	Black Angus	~ 2000	~ 6.7	AB
10	02/05/2007	Holstein	10/11/2001	5.5	BC
11	18/12/2007	Hereford	1992	13.8	AB, L-type BSE
12	26/02/2008	Holstein	21/12/2001	6.2	AB
13	23/06/2008	Holstein	22/04/2003	5	BC
14	15/08/2008	Gelbvieh X	20/03/2002	6	AB
15	14/11/2008	Holstein	01/01/2001	7.9	BC
16	15/05/2009	Holstein	2003	10	AB
17	24/02/2010	Black Angus	22/03/2004	6	AB
18	21/02/2011	Holstein	23/08/2004	6.4	AB
December	23rd, 2003:	Holstein	Found in	US, traced to	Canada (DOB: 09/04/1997, 6.7 years)

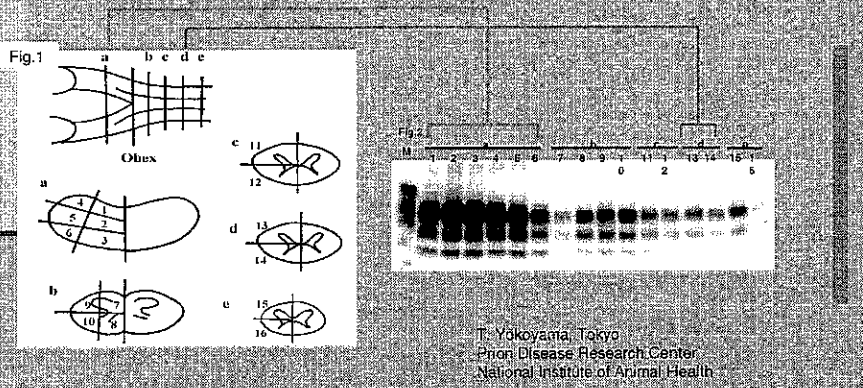


BSE RL Quality Management for surveillance

- **Validation & approval of BSE rapid tests before implementation (BSE RL & CFIA VBs)**
- **Serial release testing of kit batches by BSE RL**
- **Screening laboratories & test kit suppliers to work under standardized quality management systems (ISO) (On-site inspection by CFIA VBs)**
- **Screening laboratories to participate in proficiency panels**
- **Training & certification of analysts**



PrP^{Sc} distribution in BSE cattle



Applied Research: IHC/Histology

- Increasing sensitivity for confirmatory testing: IHC, PET-BLOT
- Antibody evaluation
- Detection kit comparisons/evaluations
- New equipment evaluation

Applied Research: Molecular Tests

- Transfer & optimization of ultra sensitive tests for prion diseases (RT-QIK)
- Determination of screening/confirmatory test diagnostic & analytic sensitivity
- Molecular characterization of Canadian BSE cases (classical & atypical)
- Confirmatory test refinement including evaluation of newly available equipment

Basic Research:

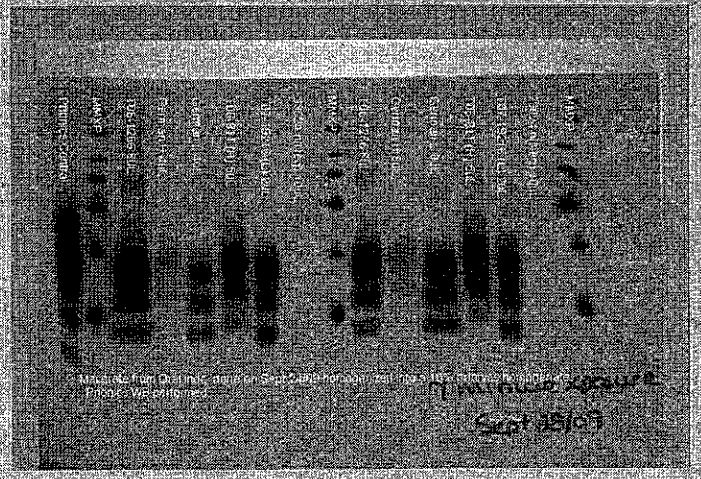
- CWD Risk Assessment (Primate inoculation) (APRI; Germany/UofC)
- Risk Assessment of atypical BSE in cattle (HC, ALMA/APRI)
- BSE biomarker in bovine urine (PHAC)
- Prion decontamination via composting (lead: Ag Canada)

Atypical BSE

Significance ?

	L-Type	H-type	Total
Canada	1	1	2
Denmark	1	0	1
France	10	11	21
Germany	1	2	3
Ireland	0	1	1
Italy	3	0	3
Japan	1	1	2
Netherlands	2	1	3
Poland	8	2	10
Spain	3	6	9
Sweden	0	1	1
Switzerland	0	3	3
UK	0	3	3
USA	1	2	3

Classical & Atypical BSE



Intra-species transmission (i.c.)

- L-type BSE: transmissible to cattle: ~ 11mpi (Holstein & Hereford/Angus X); ~ 17 mpi (Brown Swiss). Clinical disease: apathic or nervous. Glycoform ratios like type-2 sCJD. Tg bov (145-185 dpi)
- H-type BSE: transmissible to cattle: ~ 12 mpi (Holstein & Hereford/Angus X)). Clinical disease: nervous or apathi.. Tg bov (250 dpi)
- C-type BSE: transmissible to cattle: ~ 20 mpi (diff. breeds). Clinical disease: ataxic. Tg bov (230 dpi)

Inter-species transmission

- L-type BSE: non-human primates (17 mpi: microceps, 21 mpi: macaques). 2D fingerprints like sCJD (MV-2, VV-2 subtypes).Tg hu (~ 400 dpi), more infectious than C-type BSE. Hamster: 217 dpi (2. passage) → electrophoretic profile: C-type BSE. Tg ov: electrophoretic profile of C-type BSE.
- H-type BSE: non-human primates (37.5 mpi). WT mice, Tg hu: similar to sCJD MM1.
- C-Type BSE: non-human primates (Cynomolgus: 40 mpi, 63 mpi). Tg hu: WT mice.

Atypical BSE experiments:

- Intracranial challenge (Canadian C-, H- & L-type BSE) (n=7). 1ml, 10% homogenate
- Peroral challenge (German H- & L-type BSE) (n=17). 100gr

Hereford Angus X (SPF)

6 months old calves (n=14)

13 months old steers (n=10)

What is atypical BSE ?

BSE in older animals (after infection early or late in life) ?

Several, unrelated strains of prion disease with longer incubation periods than c-type BSE ?

Spontaneous/sporadic disease (sCJD) ?

Normal Maturation/Aging of cows ?

Infection with other prion diseases (Scrapie) ?

Origin of c-type BSE ?

Experimental approach:

Octapeptide repeat genotypes:
5/5 (n=2), 5/6 (n=3), 6/6 (n=12)

Prnp gene: done for homozygous, cloning & sequencing on-going for heterozygous

Titration of challenge material:TgbovXV

Monthly Sampling (blood, urine, feces)

Monthly clinical examination (Ueli Braun)

Clinical signs indicative for BSE:

i.c. L-type (acoustic stimuli): 11-12 mpi
i.c. H-type(tactile stimuli): 11 mpi
i.c. C-type: 16–18 mpi
p.o.H-type calf: 18 mpi
p.o.L-type calf: 18 mpi

Principle of BSE Ante Mortem tests:

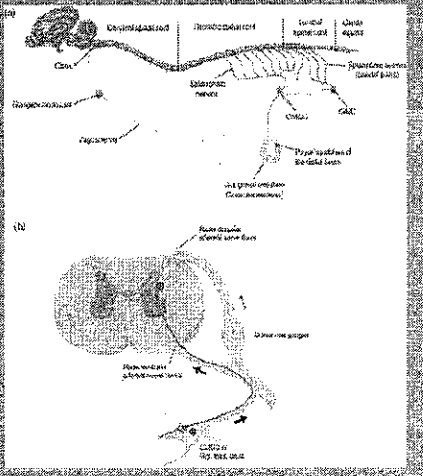
Disease-specific or direct tests: (PrP^{sc} detection). Challenge is low level of PrP^{sc} but high level of PrP^{C} in blood

Surrogate markers or indirect tests (detection of molecular changes or other parameters)

Live animal/ ante mortem test for BSE

Spread & Distribution of BSE

(Ch. Hoffmann et al. 2011)



EFSA requirements for Ante Mortem Tests:

For consumer protection:

Performance should not be statistically inferior to that of currently approved post mortem tests (>98.5%)

Performance should be capable of detecting infected animals earlier in the incubation period

Evaluation protocol for ante mortem BSE tests

Diagnostic Sensitivity (54 reference samples of 53 confirmed BSE positive animals)

Diagnostic Specificity (558 samples of 488 animals):

CAT1NEG (450 samples of 386 BSE negative NZ animals)

CAT2 SUSP (70 samples of 67 clinically suspicious animals)

CAT3 DD (38 samples of 35 animals with other diseases or infections)

Detection of BSE at early stages (3 p.o.infected cattle, 1 unchallenged control, 24, 30, 34 & 40 mpi. n=16)

Evaluation exercise for ante mortem BSE tests

EC call for expression of interest (2007, ante- & post mortem tests)

Target: tests in advanced stages of development or available for use

Request: to assure that tests are available on a non discriminatory basis following evaluation

Assessment of application dossier (15 external scientists): scientific basis of test, available experimental evidence, practicality of sampling & testing procedures, stage of development of test

6 different tests, 6 different parties

1 test was selected for evaluation exercise

Samples of CAT3 DD

Symptoms/Disease/Infections	
Depression caused by purulent sinusitis	Sinusitis-meningits
CNS caused by severe metabolic acidosis	Meningitis
Dysphagia-Bulbar paralysis	Listeriosis
Parease hind quarters & Enteritis	Botulism
Central Nervous Symptoms	Enteritis
Impossible to stand up	Tetanos
Excitation-fever-slight, dysphagia	Dystocia, paralysis
Dysphagia-fever-Listeriosis-brain abscess	Ataxia
Spasms due to spinal cord lesion	Hypocalcemia
Paresis/depression/hypokalemia/alkalosis	Malignant catarrhal fever

Ante mortem test: conclusion

Post mortem tests suffice for epidemio-surveillance

Use of ante mortem test for BSE requires a policy change

BSE Reference laboratories:

Drs. S. Czub, C. Graham - pathologists,

R. Clark, S. Dudas, R. Hilton, R. Quaghebeur, K. Santiago-Matteo, M. Snodgrass, J. Yang: rapid dx tests & molecular work

T. Ambagala, K. Colwell, Y. Fang, T. Pickles: IHC, histopathology