European Commission

Better Training for Safer Food

Initiative

TSE/BSE: an introductory overview

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Consumers, Health And Food Executive Agency

European Commission

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Chapter 1. WHAT ARE TSEs?

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What are TSEs?

TSEs are neurological diseases, caused by proteinaceous infectious particles, the '**prions**'

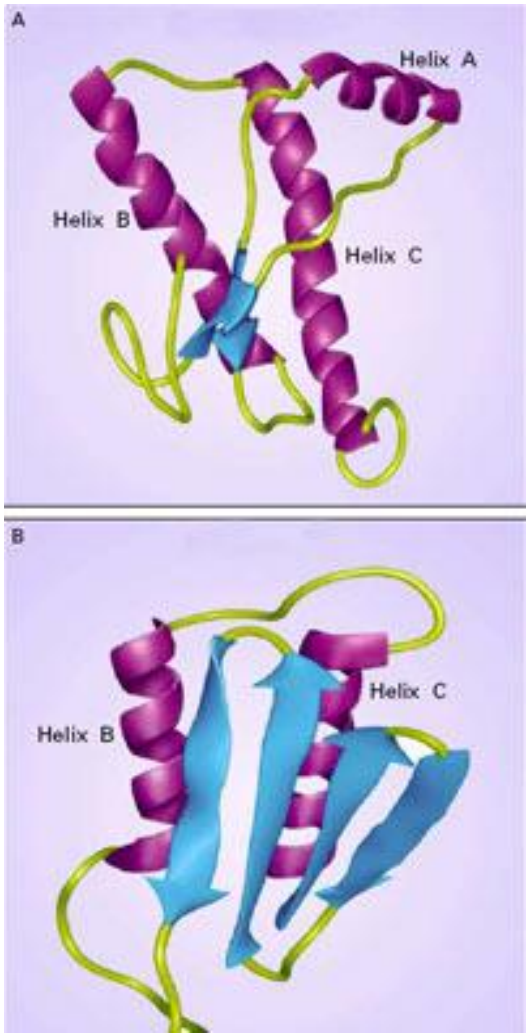
Misfolding of physiological form of PrP, PrP^{c} (α-helix), to disease-associated form, **PrP^{res}** (β-sheet)

Function of PrP still unknown but **PrP^{res} in brain is a consistent infectivity marker**

Existence of **multiple protein conformations may explain different strains**

PrP **gene sequence may influence susceptibility** to disease (intra- and inter-species)

Infectious, spontaneous (sporadic), hereditary forms of prion diseases occur in animals or humans



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Animal Prion Diseases

Scrapie - sheep, goats

Chronic Wasting Disease (CWD) - deer, elk, moose

Bovine Spongiform Encephalopathy (BSE) - cattle

Transmissible mink encephalopathy (TME) - mink

Feline spongiform encephalopathy - large and domestic cats

Spongiform encephalopathy of captive ungulates - exotic hoof-stock in zoological parks

Human Prion Diseases

Zoonotic evidence only for ** Variant CJD or Human BSE

Sporadic

- **Creutzfeldt-Jakob disease (CJD) (1 per million per year worldwide)**

Familial (genetic)

- **Familial CJD**
- **Gerstman-Straussler-Scheinker Syndrome (GSS)**
- **Fatal Familial Insomnia (FFI)**

Acquired by transmission

- **Kuru: endocannibalism Papua New Guinea 2700 → SRM**
- **Iatrogenic CJD (neurosurgical instruments, dura mater grafts, HGH) > 405**
- ****Variant CJD (vCJD) or Human BSE 228 patient mortalities (worldwide) 1 in 2000 carriers (UK)**

→VPSPr (Variably protease-sensitive prionopathy) Gambetti 2008

Transmission Within Species

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COMMON

vertical and horizontal

in utero, fetal fluids, fetal membranes

horizontal

Oral (urine, feces, or blood?)

UNCOMMON

Foodborne
Direct only through bite wounds

Foodborne (MBM)
No direct transmission
from cow to cow

Foodborne, blood,
tissue transplant, HGH, instruments

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Species Barrier Concept

Transmission within a species may occur readily

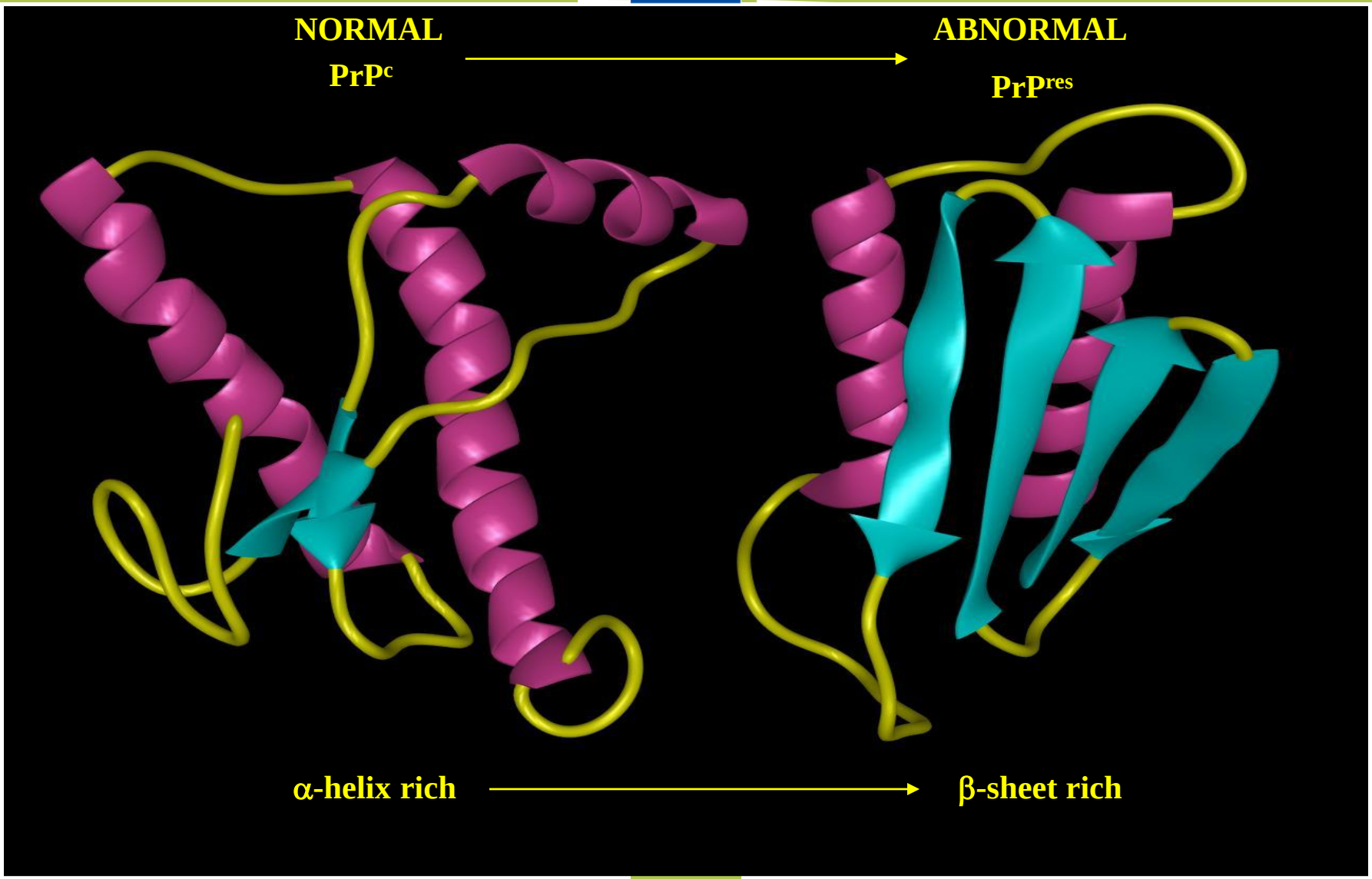
Barrier between species limits transmission

- **Inefficient transmission**
- **Extended incubation times**
- **Low or non-existent rate of disease**

Serial passage

- **Required to overcome species barrier**
- **Progressive reduction in incubation time**
- **Increased rate of disease**

Transmissible Spongiform Encephalopathies~Prion Disease



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PrP Conversion

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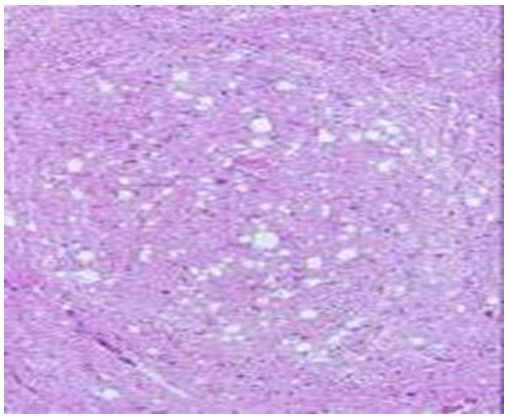
What are TSEs?

Transmissible (or sporadic/genetic)

Spongiform lesions in CNS (or not)

Encephalopathy – lesions primarily found in the brain (and/or spinal cord, tonsils, lymph nodes, gut, liver, kidneys, adrenals, eyes…)

Current definitions now all include the presence of PrP^{res}



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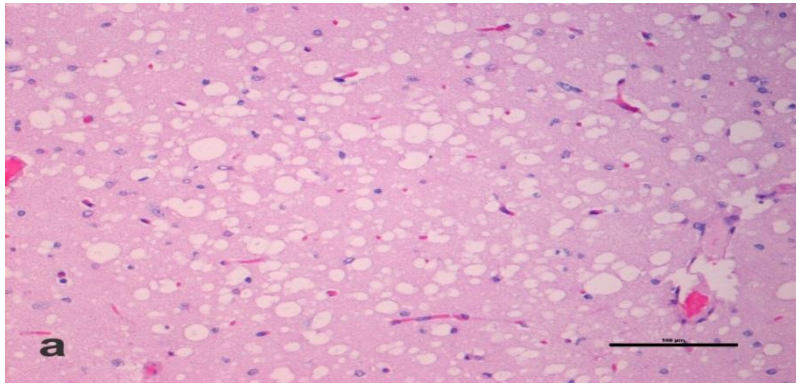
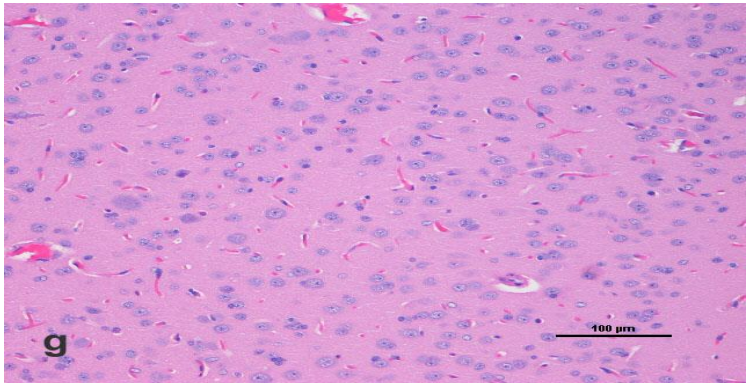
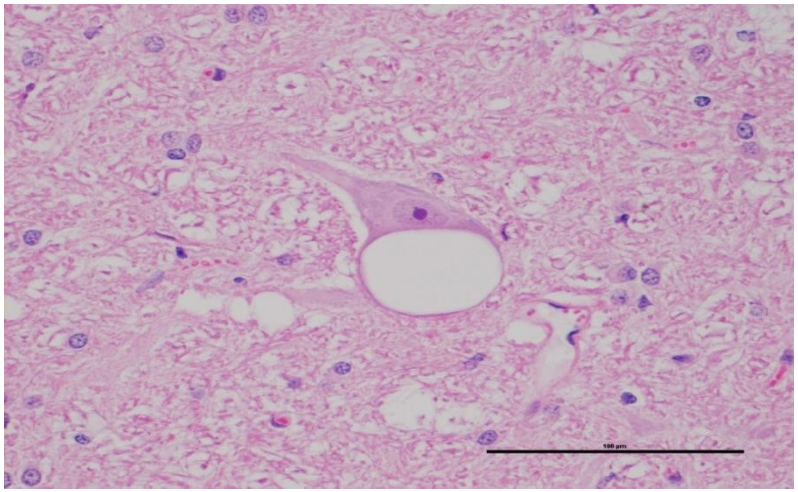
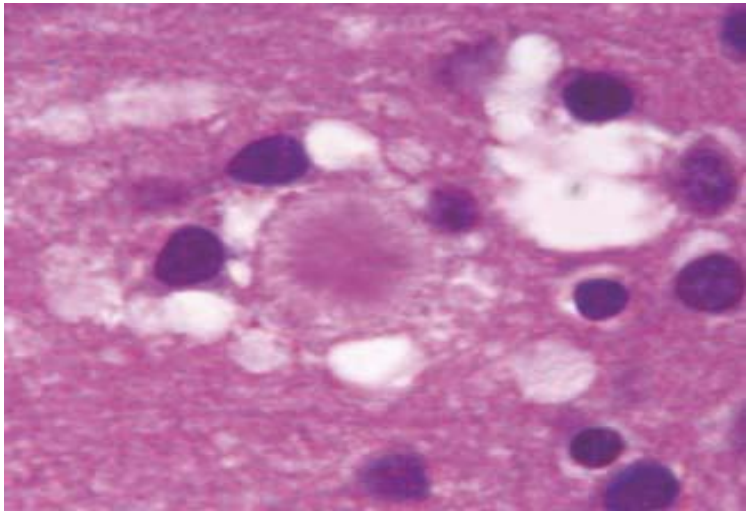
Histology









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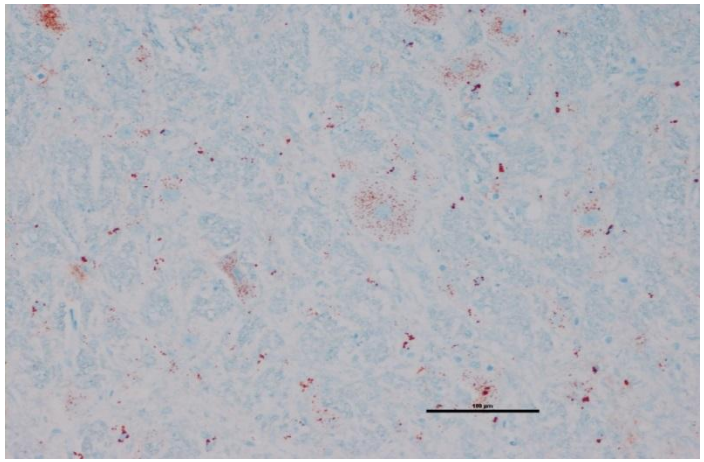
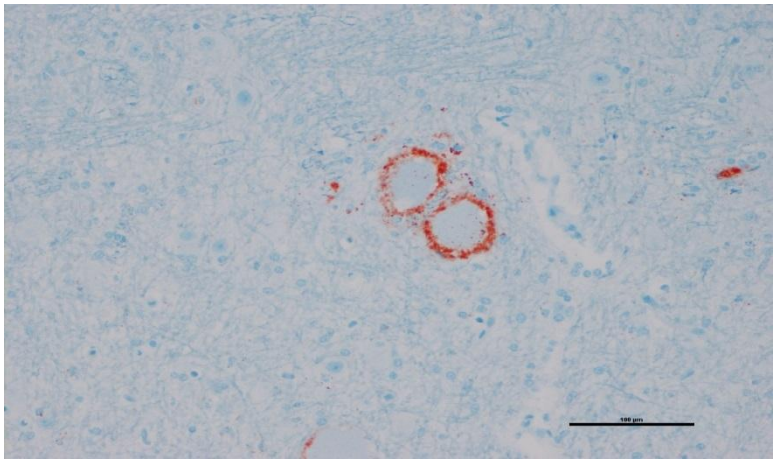
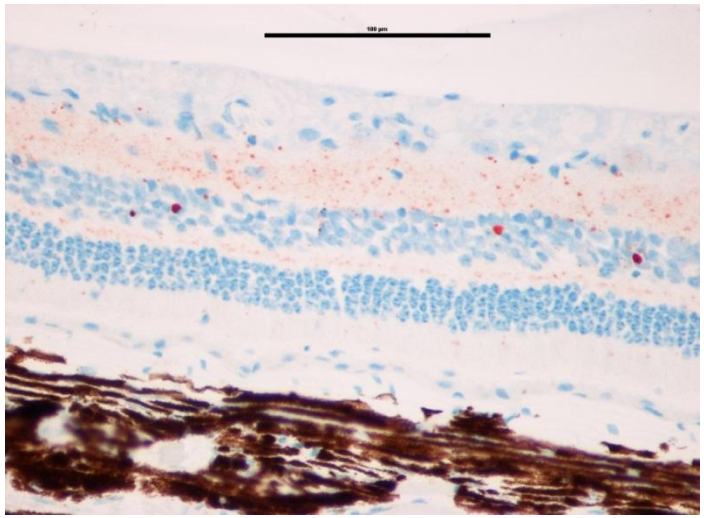
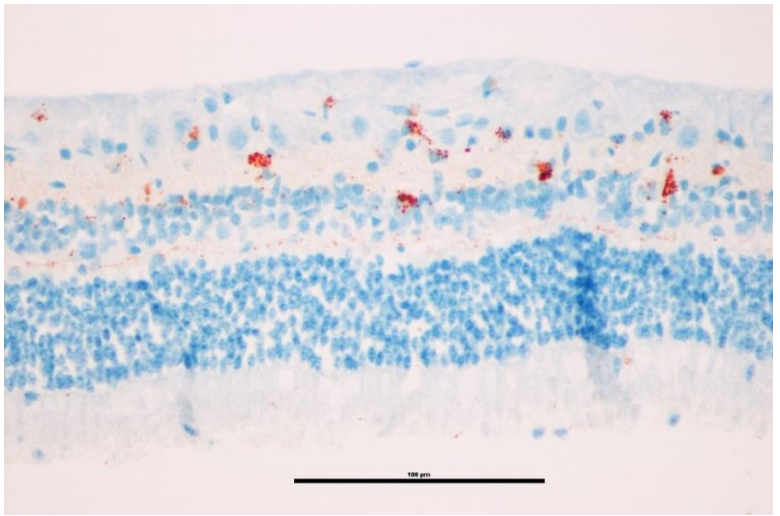
Immunohistochemistry









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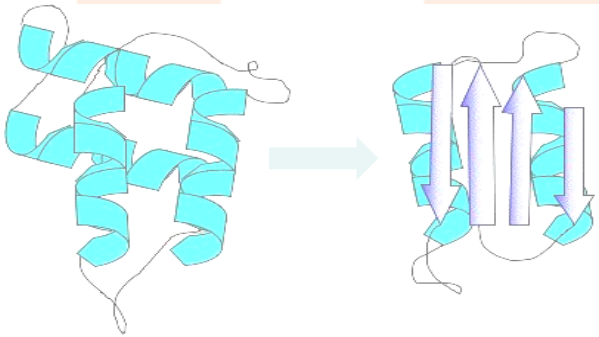
What is the BSE/TSE hazard and its characteristics?

The BSE-TSE Agent.

<u>Nature of the BSE-TSE Agent is not fully understood yet</u> but, even though not identical, a proteinaceous entity* (PrP^{res}) is routinely used as a marker for infectivity.

256 amino acids, 26-32 kilodaltons



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BSE/TSE hazard and its characteristics

BSE, is part of a group of transmissible brain affections or prion diseases, characterised by:

Usually, "spongiform" degeneration of neuronal cells.
Occurrence in man and animal
Usually, a fatal outcome
Long incubation period
No **apparent** immune reaction.

Characterised by the transformation of normal brain protein (PrP^{c}) into an abnormal protein (PrP^{res}) or *prion* which is routinely used as a marker for infectivity.

Prion **usually** resistant to (at a variable degree…):
- Heat / Ultraviolet light and ionising radiation
- Enzymes
- Chemical substances

PRIONS HAVE A VERY HIGH AFFINITY FOR STAINLESS STEEL→ surgical instruments + prototype test for Human BSE using stainless steel powder

Inactivation of TSE agents

→ Also depending on strain!

Physical methods

Irradiation (ionizing, UV, microwave) :		*little effect*
Dry heat : 360°C 1h and 600°C 15 min:		*partial survival*
Autoclaving : conflicting data!		
Gravity displacement : 132°C 1h :		*residual infectivity*
	132°C 90 min :	***inactivated***
	134°C 30 min :	*5.3 logs reduction*
Porous-loud :	134°C 18 min :	***inactivated***
	138°C 1h :	*residual infectivity*

→ *increased temp = increased thermostability?*

Inactivation of TSE agents

Chemical methods

Acids and bases : pH 2-10 1h : *little effect*
pH 14 2M NaOH : *5 logs reduction*
→combination GD 121°C 1h 1M NaOH : ***complete inactivation***

Alkalyting agents : formaline, glutaraldehyde, acetylethyleneimine,bèta-propiolactone, ethylene oxide : *no effect or increased!*

Detergents : SDS+boiling or sarkosyl : *some effect*

Halogens : Sodium hypochlorite (25.000ppm chlorine) 1h : ***effective***
Sodium iodide 2% : *little effect*

Organic solvents : acetone, chloroform, ethanol, phenol, hexane, perchlorethylene, petroleum : *little effect*

Oxidizing agents : chlorine dioxide, hydrogen peroxide, peracetic acid *:* *little effect*
Salts : sodium periodate, potassium permanganate: *contradictory results*

Chaotropes : 4M GdnSCN or GdnHCl : *some effect*

Proteolytic enzymes : pronase and proteinase K: *some effect*

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Hazard Characterisation

In general, there is a dose-response relationship in experimental TSE infectivity (incubation time)

It is assumed that a TSE human-animal species barrier exists, which would affect the efficacy of TSE transmissibility to humans

However, both dose-response relationship in humans and the bovine-human species barrier can only be roughly estimated using animal models

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Hazard Identification

What defines a particular TSE Agent?

1. **Biochemical characterisation (orientative)** Western blotting, ELISA
2. **Biological characterisation**: Inoculation in mice (RIII, C57Bl, VM, Tg mice) (not routinely applicable, laborious technique).

→Transmissibility;
→Lesion profile;
→Incubation period;
→Western blotting characterisation. Topology and quality of PrP^{res} deposition.

In 1996, a new form of CJD, named variant CJD or human BSE, was identified in humans and it was demonstrated to be caused by the agent that causes BSE in cattle.

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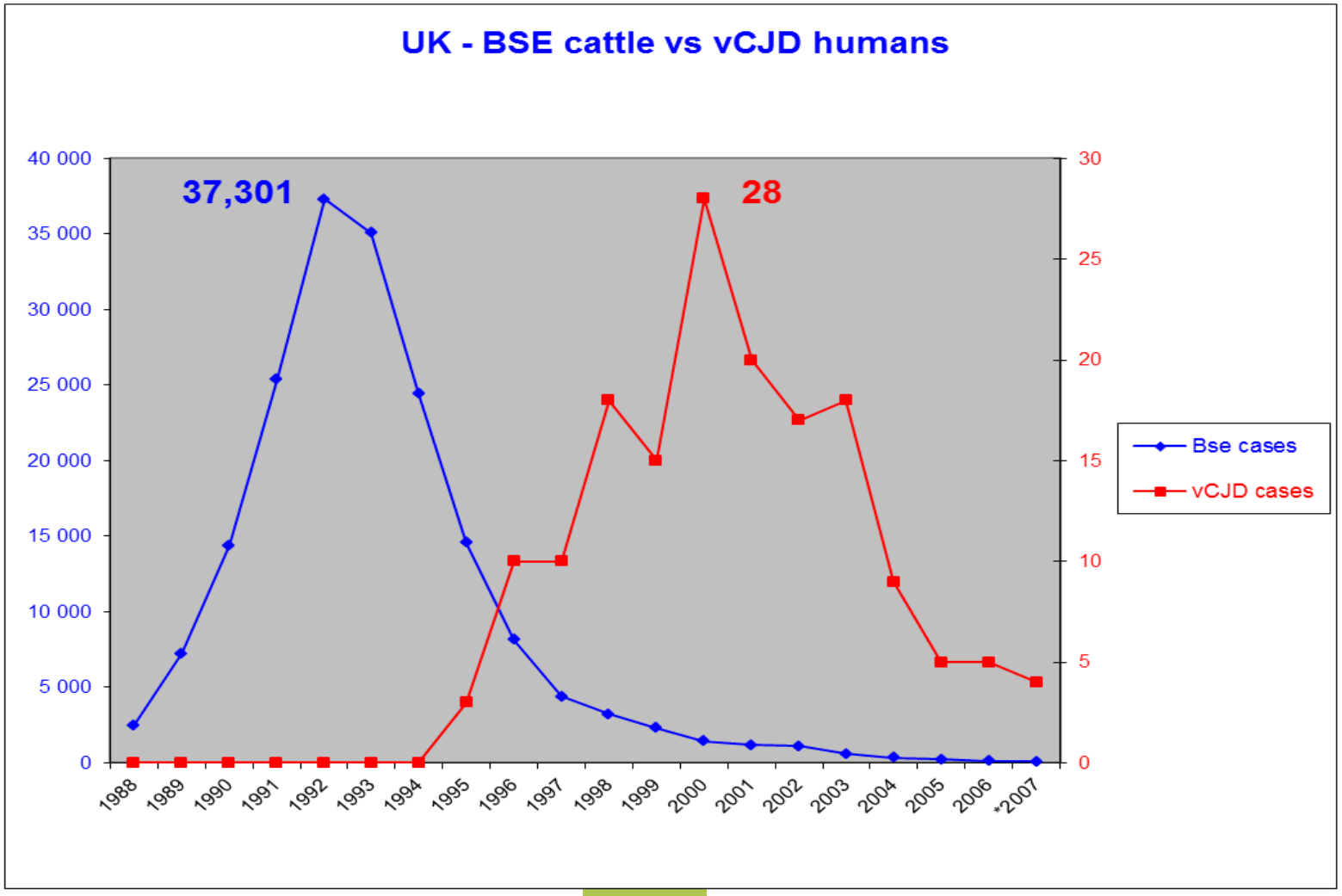
Hazard Identification

3 million estimated UK cattle BSE →176 UK primary human BSE



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Hazard Identification

The origin of BSE? UNKNOWN…

Conversion of physiological PrP^{C} into the abnormal PrP^{res} or Prion.

Main hypotheses:

- **Spontaneous occurrence - never proven (L-type?)**
- **Scrapie transmission to bovines - not experimentally shown**
- ***1996 Organophosphates? (Phosmet)?***
- ***1996 Spiroplasma?***
- ***2001 Microbacteria from meteorites froling the earth? Cambridge, Wickramasinghe and Hoyle***
- ***2003 Acinetobacter? Veterinary and Immunopathology, Wilson et al***
- ***2005 Cadavers from the Ganges? The Lancet, Shankar and Satischandra***

Only commonly accepted:

- **it appeared somehow in UK, already in the 70s**
- **it was distributed from there via export of feedstuffs and of infected cattle**

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Hazard Identification

Transmission of BSE in cattle:

MBM (never experimentally reproduced) aggregate associated infectivity -> only few animals per herd **: what is the field CoID50 (Experimentally between 1mg and 0.1 mg fresh BSE brain)**

Horizontal transmission - NO!?

Vertical transmission - HOW?

Oral – via feed or via injection of infective tissue

Semen - not

Embryos – unlikely

Other ??

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Chapter 2. THE SCIENCE-BASED EU TSE LEGISLATION AND RELATED LEGISLATION

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The Science-based EU TSE legislation and related ABP legislation: risk analysis

All EU TSE legislation has to be based on Science

Scientific Opinions of EFSA

The TSE Agent behaves both like a microbiological and a chemical contaminant

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Risk Analysis framework in the EU

Question?

- **European Commission**
- **European Parliament**
- **Member States**
- **EFSA ("self mandate")**

efsa
European Food Safety Authority

Risk Assessment

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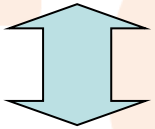
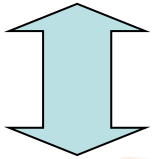
Risk Analysis framework in the EU

Independent

BIOCONTAM Panel of Scientific Experts



Working Groups of Scientific Experts



EFSA BIOCONTAM Staff

BIOCONTAM Scientific and Administrative Secretariat

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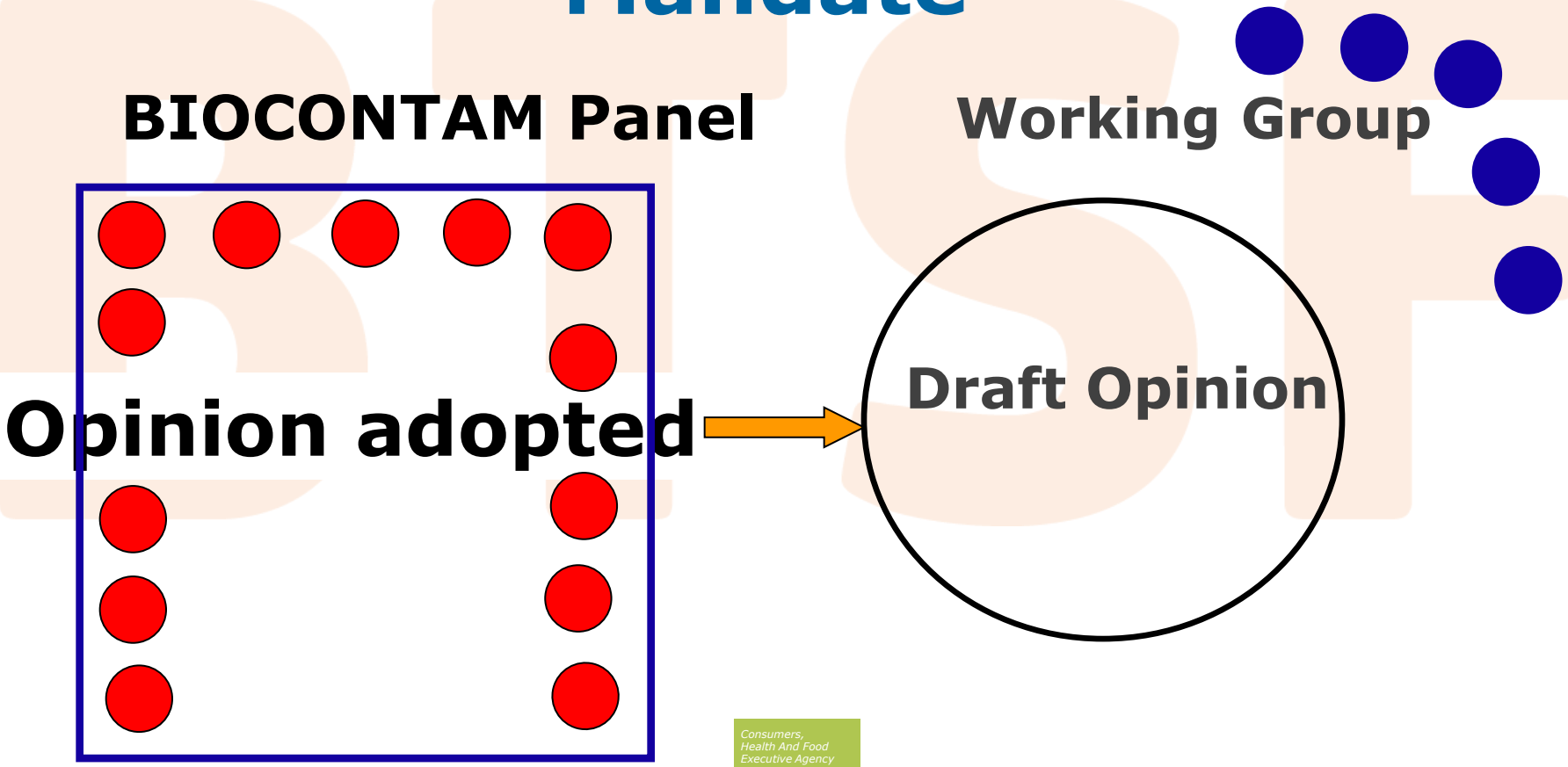
Risk Analysis framework in the EU

Mandate

BIOCONTAM Panel

Working Group



Risk Analysis framework in the EU

Question?

- European Commission
- European Parliament
- Member States
- EFSA ("self mandate")

Risk Assessment

Opinion

Risk Management

- Consumers
- Media
- Industry
- Professionals

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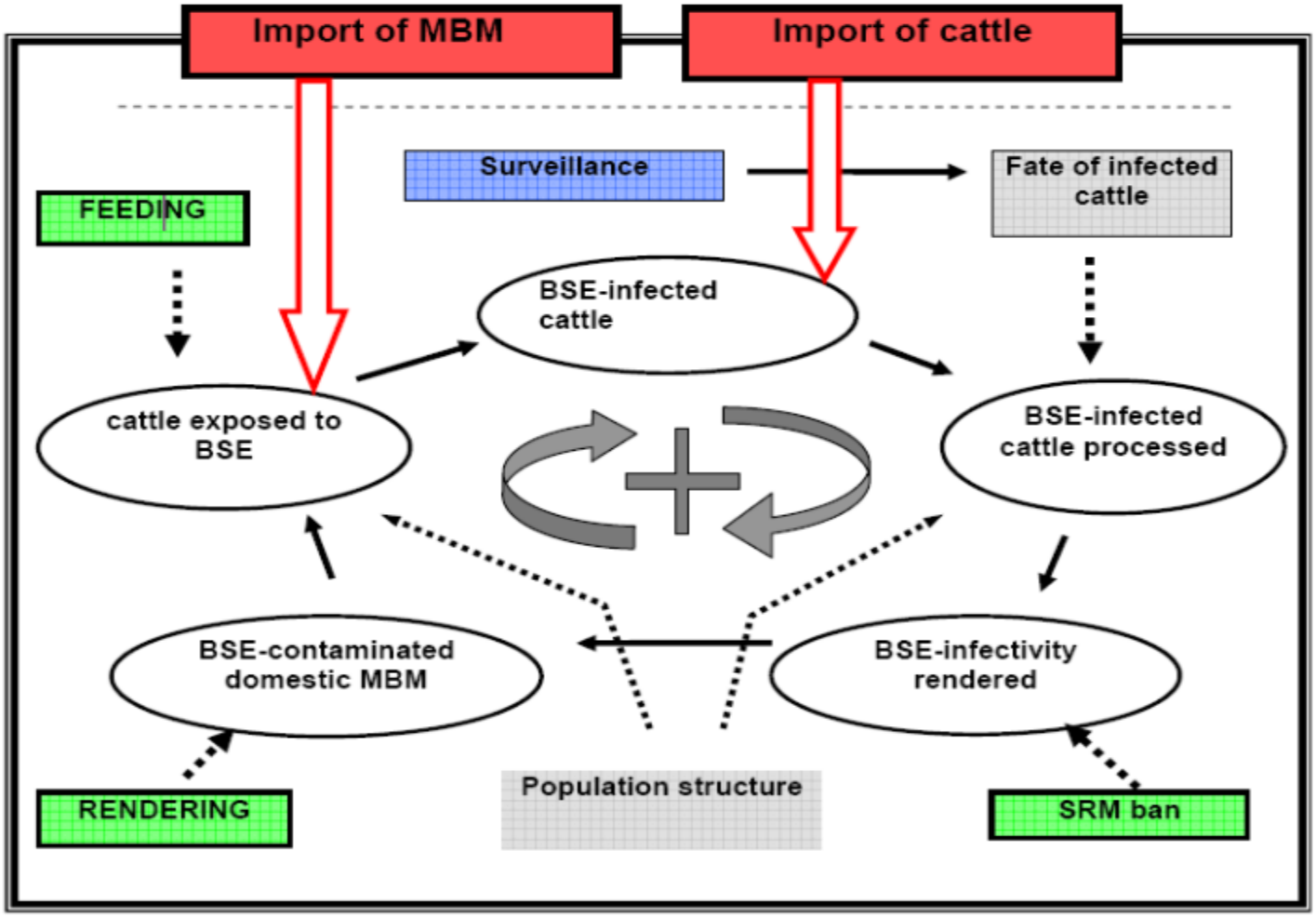
Import of MBM
Import of cattle
Surveillance
Fate of infected cattle
FEEDING
BSE-infected cattle
cattle exposed to BSE
BSE-infected cattle processed
BSE-contaminated domestic MBM
BSE-infectivity rendered
RENDERING
Population structure
SRM ban

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TSE regulation 999/2001

- **Sets out parameters for TSE suspects and eradication measures and definition of SRM – category 1 ABPs (Art 8 of 1069/2009)**

- **Rules for export of SRM set down in TSE rules**

- **Prohibitions on feeding – complements restrictions in Art 11 of 1069/2009**

- **TSE roadmap - stepwise amendments relaxing TSE rules e.g. on feeding prohibitions**

Animal By-Products legislation (1069/2009 and 142/2011)

→Boundaries of ABP legislation (1069/2009 and 142/2011) with following legislation:

- **TSE regulation- 999/2001**
- **Food hygiene legislation -852/2004 and 853/2004**
- **Feed legislation – 183/2005 (hygiene) and 767/2009 (placing on market)**
- **Various legislation on cosmetics, medical devices, veterinary / medicinal legislation**
- **Environmental legislation (main focus) - Waste Framework Directive 2008/98 and Waste Incineration Directive 2000/76**

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Chapter 3. EXPOSURE AND ZOONOTIC POTENTIAL OF ANIMAL TSEs

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VARIANT CREUTZFELDT-JAKOB DISEASE OR HUMAN BSE
CURRENT DATA (2014)

Estimation: 1/2000 subclincal infected with BSE (UK)

Confirmed cases: 228 from 12 countries

176 United Kingdom, 27 France, 5 Spain, 4 Ireland, 3 United States, 3 the Netherlands, 2 Portugal, 2 Italy, 2 Canada, 1 Japan, 1 Saudi Arabia, 1Taiwan

COUNTRY	TOTAL NUMBER OF PRIMARY CASES (NUMBER ALIVE)	TOTAL NUMBER OF SECONDARY CASES: BLOOD TRANSFUSION (NUMBER ALIVE)	RESIDENCE IN UK > 6 MONTHS DURING PERIOD 1980-1996
UK	172 (4)	4 (0)	176
France	27 (0)	-	1
R of Ireland	4 (0)	-	2
Italy	2 (1)	-	0
USA	3 (0)	-	2
Canada	2 (0)	-	1
Saudi Arabia	1 (0)	-	0
Japan	1 (0)	-	0
Netherlands	3 (0)	-	0
Portugal	2 (0)	-	0
Spain	5 (0)	-	0

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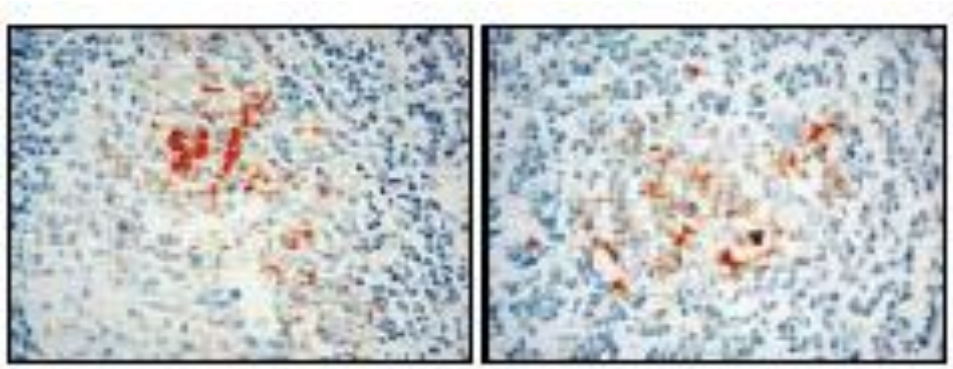
PrP^{res} in UK appendices: implications for prevalence of subclinical or preclinical Human BSE infections



Over 32 000 anonymous appendix samples →one in 2000 people are likely to be carriers.

No particular age group or geographic region affected, no susceptible genotype of patients was identified.

A higher proportion of valine homozygous (VV) genotype in codon 129 of the gene encoding the prion protein (PRNP) compared with the general UK population. This also differs from the 177 patients with Human BSE, all MM (oral exposure BSE infected meat products)

What is real risk carriers pose of transmitting the disease by blood transfusion or surgery?

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Transmission of TSE by blood transfusion

SHEEP

Around 20% transmission of infectivity by transfusion of whole blood or buffycoat cells from BSE and Scrapie preclinical and clinical donor incubating transfused into recipient sheep

HUMANS

- **Infectivity in erythrocytes, leukocytes, and plasma in vCJD or human BSE**
- **Infectivity levels comparable to those reported in various animals with TSEs**
- **In the United Kingdom, 4 vCJD transmissions from 18 donors who later had positive test results for vCJD**

Exposure

To what extent are consumers exposed to the BSE Agent?

Pathogenesis studies serve as the basis for the identification of potentially infectious cattle material (mainly CNS).

Titration of this material can help to quantify the infectious load and thus the exposure risk.

Based on these, the Specified Risk Materials are defined. Its removal from the human food and animal feed chains is the most efficacious measure to decrease the exposure risk (estimated to be at least 95% of the total infectivity).

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Exposure Assessment

Pathogenesis studies serve as the basis for the definition of (SRM)
Assumption:100 mg =1 $CoID_{50}$ but recent data 1 - 0.1 mg = 1 $CoID_{50}$

Tissue	Infectivity density ($CoID_{50}$/g)	Weight (kg) per 537 kg animal	Cattle oral ID50 per BSE Case	% of total infective load per animal	Cumulative load
Brain	10	0.5	5000	64.1 %	64.1 %
Spinal cord	10	0.2	2000	25.6 %	89.7 %
Trigeminal ganglia	10	0.02	200	2.6 %	92.3 %
Dorsal root ganglia	10	0.03	300	3.8 %	96.1 %
Ileum	3.20 E-02	0.8	26	0.3 %	99.4 %
Spleen*	3.20 E-02	0.8	26	0.3 %	99.7 %
Eyes	3.20 E-02	0.1	3	0.04 %	99.74 %

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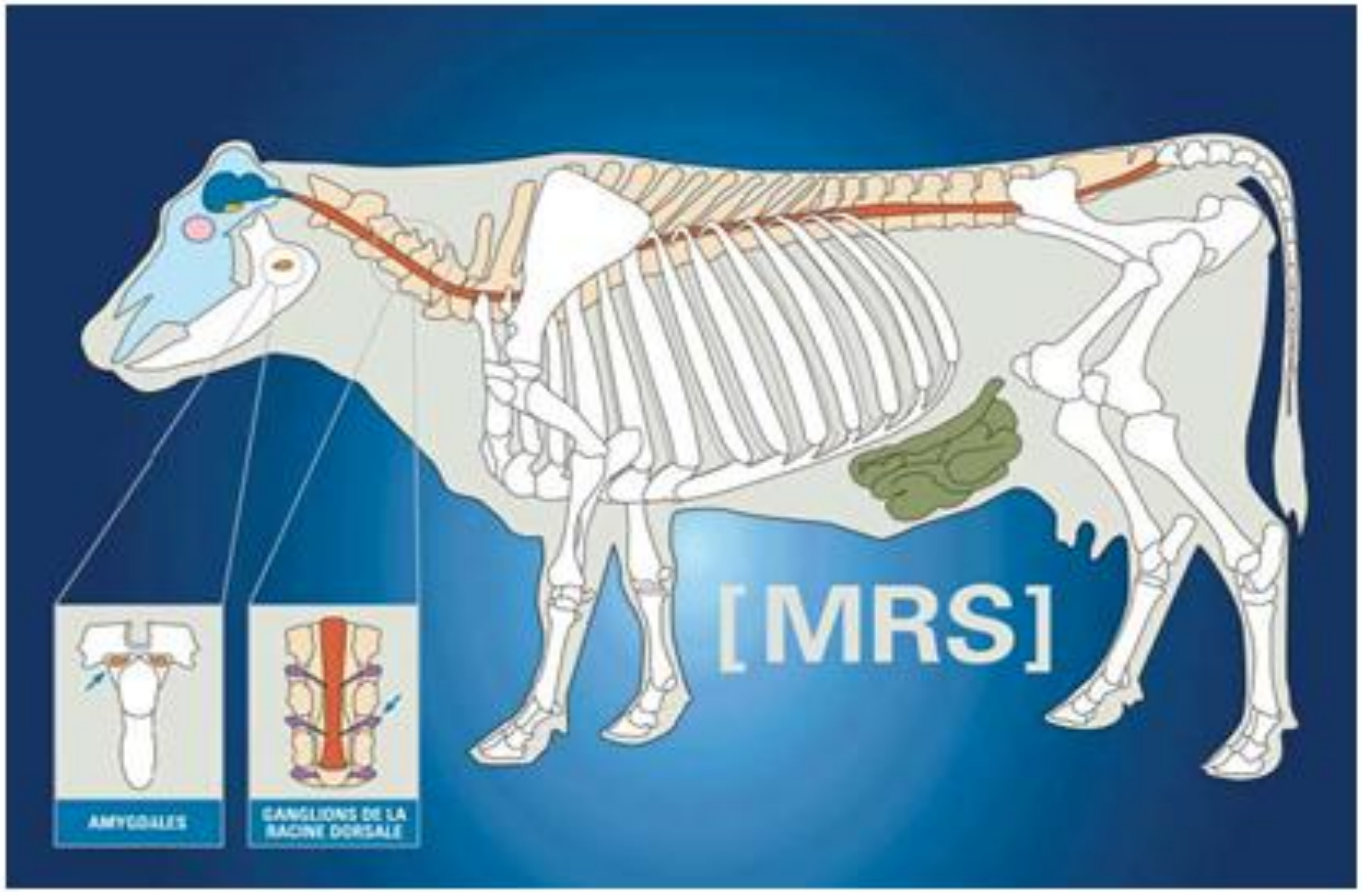

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Exposure Assessment

Where lays the residual Classical BSE exposure risk?

Clinical BSE cases: Should not enter the human food chain (*antemortem* veterinary inspection).

Pre-clinical BSE cases: Eclipse phase of several years after infection (distal ileum >> sympathic/parasympathic nerves >> CNS): risk mainly from end-stage incubating animals.

Exposure only through potential **cross contamination** during slaughtering process and hypothetical residual infectivity left in lymphoid (and nervous?) tissue.

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Exposure Assessment: Main TSE monitoring uncertainties

Atypical BSE (H or L-type): Efficiency of the current TSE monitoring system? No clinical signs, > 10 years of age, no healthy slaughter testing

The impact of TSE testing policy on TSE monitoring in cattle: Considerations on sensitivity, active and passive surveillance, early detection.

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Negligible BSE risk

Members recognised as having a **negligible BSE risk** in accordance with Chapter 11.5 . of the *Terrestrial Code* :

Argentina	Iceland	Panama
Australia	India	Paraguay
Austria	Israel	Peru
Belgium	Italy	Singapore
Brazil	Japan	Slovenia
Chile	Netherlands	Sweden
Colombia	New Zealand	United States of America
Denmark	Norway	Uruguay
Finland		

Controlled BSE risk

Members recognised as having a **controlled BSE risk** in accordance with Chapter 11.5. of the *Terrestrial Code* :

Bulgaria	Germany	Malta
Canada	Greece	Mexico
Chinese Taipei	Hungary	Nicaragua
Croatia	Ireland	Poland
Costa Rica	Korea (Rep. of)	Portugal
Cyprus	Latvia	Slovakia
Czech Republic	Lichtenstein	Spain
Estonia	Lithuania	Switzerland
France	Luxembourg	United Kingdom

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EUROPE: OIE Member Countries' official BSE risk status map

Last update May 2013

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Exposure Assessment

Food processing is **not** assumed to affect infectivity potential, but can only dilute this (like chemical contaminant).

Consumer habits do **not** clearly address increased exposure in particular sub-populations.

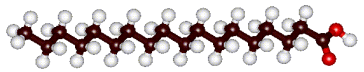
Consumer cooking practices are **not** assumed to affect infectivity if present

Variety of usage of Animal By Products (examples)

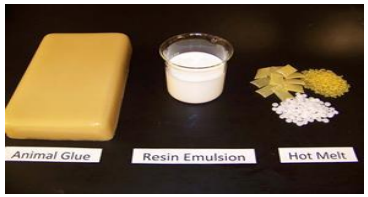
OF/SI: BSE/TSE prions in soil and plants relatively stable against protein-denaturing "influences"

Shopping bags–slip agent from animal fat

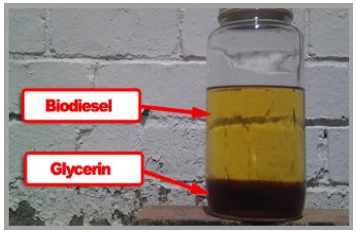
Tyres & fireworks-animal based stearic acid



Violins & pianos–animal glue



Cosmetics – glycerin



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Zoonotic potential of animal TSEs

Zoonotic risk of other TSE Agents?

Scientific data:

Small ruminants TSE Agent transmitted to cynomolgus and marmoset monkeys (i.c.)…

L-Type BSE transmission rate in Tg Hu mice higher than Classical BSE (i.c.)…

CWD transmitted to non-human primates (i.c)…

…The "*Howevers*",

Does the susceptibility of the animal models resemble that of the humans (dose-response and species barrier)?

Does the experimental exposure route resemble the natural human exposure route?

No evidence of epidemiological link!

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Zoonotic potential of animal TSEs:
Main uncertainties

1. The nature of the infectious agent;
2. The global distribution of animal and human TSEs;
3. The relative efficiencies of transmission between and within species;
4. The degree of confidence in case recognition in the situation where not all aspects of the case definition are met;
5. The origin of BSE;
6. The origin of sporadic CJD and Alzheimer;
7. Why procedures that normally denature proteins can only reduce but not totally eliminate infectivity";
8. The function of the PrP^C protein;
9. The presence of a zoonotic TSE strain in small ruminants;
10. There is uncertainty on whether "prions being retained with or without replication in the digestive and other tissues of non-mammalian species fed feedstuffs containing mammalian prions".

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Atypical TSEs: unknowns

Origin:scrapie/BSE related/spontaneous?

Timing of the origin?

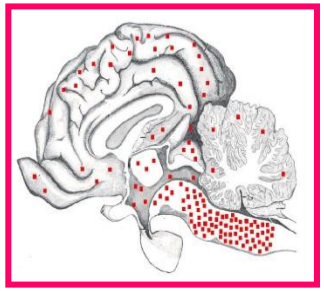
Tissue distribution of infectivity?

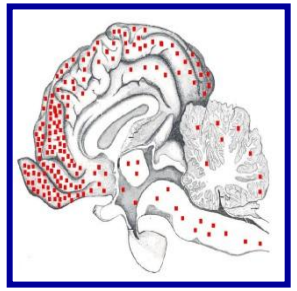
Geographical distribution?

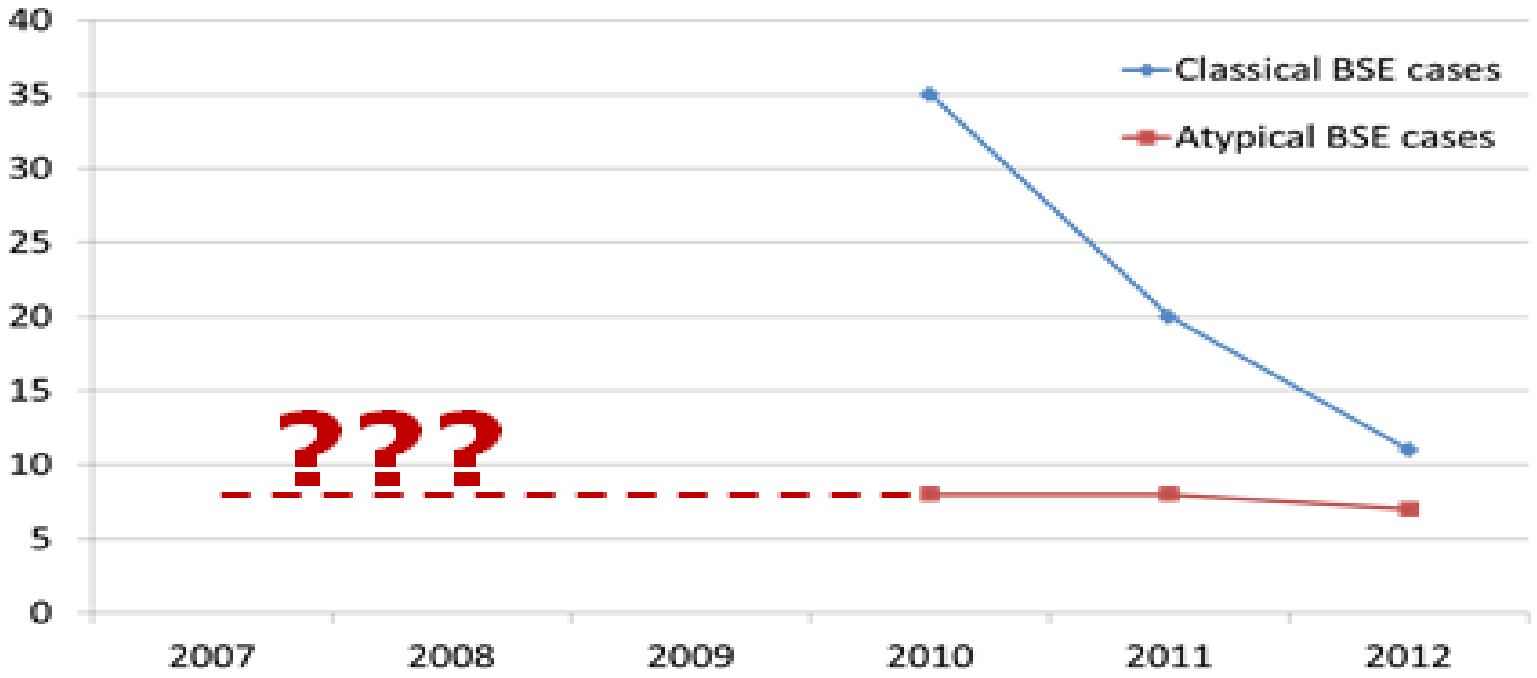
True incidence by country?

Temporal trends in incidence by country?

Phenotype in humans?






40
35
30
25
20
15
10
5
0
???
Classical BSE cases
Atypical BSE cases
2007
2008
2009
2010
2011
2012

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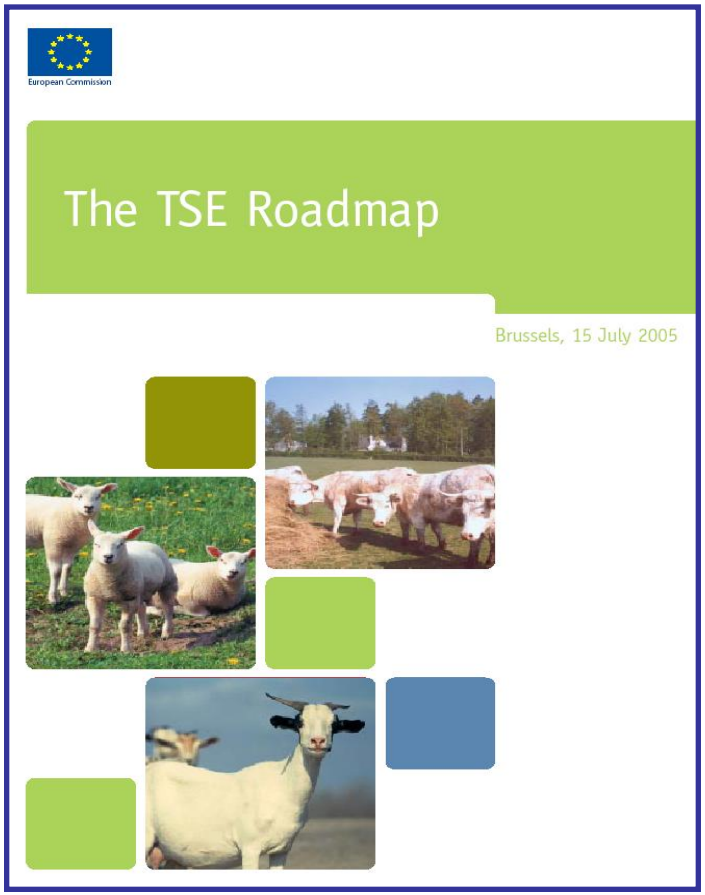
Chapter 4. TSE ROADMAP 2

Stepwise amendments relaxing TSE rules

BSE *Quo Vadis*?

European Commission

EC-DG Sanco - Risk Management



… significant decrease in the number of positive BSE cases in the EU, due to stringent risk reducing measures

…and new developments in science and technology…

…the TSE Roadmap considers possible amendments to certain BSE measures

European Commission

EFSA – *BSE* – EC/EU

↓

Science based Risk Assessments (but lot of uncertainties!)

↓

Proportionate Risk Management measures

Possible reduction of the costs for

BSE control and surveillance

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TSE Roadmap 1

- Adopted on 15 July 2005
- Reflection paper on future amendments of the TSE measures for 2005-2009
- Stepwise and **science based** approach
- Majority of short and medium term actions now achieved
- **Positive trend in BSE epidemic** has continued since then

TSE Roadmap 2

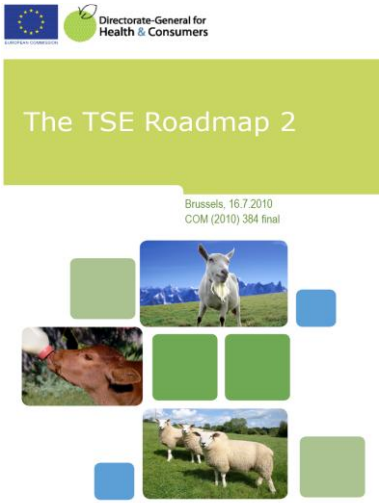
- **Communication adopted on 16 July 2010**

- **New reflection paper on future amendments of the TSE measures for 2010-2015**

- **Goal unchanged: to continue the review of the TSE measures while assuring a high level of food safety**

- **Still stepwise and science based approach (EFSA)**

- **Different topics covered: SRM removal, feed ban, BSE surveillance, TSE measures in small ruminants**



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TSE Roadmap 2: feed ban

- **Strategic goal**: to review certain measures of the current total feed ban when certain conditions are met

- Introducing **tolerance level** for processed animal proteins (PAP) in feed for farmed animals

- **Lifting feed ban provisions** for non-ruminants (pigs, poultry, fish) while avoiding cannibalism

- Conditions: control tools available, channelling

TSE Roadmap 2: feed ban

Allow non-ruminant pap for non-ruminants

- 1st step (see above) for aquafeed: 1 June 2013 (Reg. (EC) N°56/2013)
- Next steps: pigs and poultry, insects
- Principles: no TSE species, Anti-cannibalism, only cat 3
- What do we need to relax?
  - Solid basic control ABP
  - Species specific analysis
  - Species specific production/products

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Controls of the feed ban

Based on feed microscopy (Reg. (EC) N°152/2009)

Zero tolerance rule (LOD < 0.1 %)

Over 50,000 samples per year in the EU

Risk targeted controls

A new diagnostic DNA-based method which is able to detect very low level of ruminant material that may be present in feed is EC validated. That method can be used for performing routine controls on PAP and compound feed containing PAP in order to verify the absence of proteins of ruminant origin **(Reg. (EC) N° 56/2013)**

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Possible evolution of the feed ban

Lifting feed ban provisions for non-ruminants (pigs, poultry, fish) while avoiding cannibalism : Regulation 56/2013

<u>Conditions:</u> control tools available for species distinction (PCR methods) + dedicated production lines

Lifting feed ban provisions for ruminants <u>is not</u> envisaged

TSE Road map 2: BSE surveillance

- **<u>Strategic goal</u>**: to continue to **adapt the BSE monitoring system** in bovine animals with a better targeting of the surveillance activity while keeping the capacity to monitor the evolution of the epidemiological situation and to assess the effectiveness of the protective measures in place

- Options: age limit / date of birth / sample size

- Revision only allowed for Member States demonstrating a good epidemiological situation

- OIE compliance

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TSE Roadmap 2: SRM removal

- **<u>Strategic goal</u>:** to ensure and maintain the current level of consumer protection by continuing to assure safe removal of SRM but modify list/age based on new & evolving scientific opinions

- **EFSA opinions**: crucial role but quantitative or semi- quantitative approach needed

- **Alignment with OIE rules** desirable

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Proposed roadmap for SRM and Atypical BSE

- **To collect additional data in view of increasing knowledge and understanding of atypical BSE**

<u>In the meantime</u>:

- **To provisionally maintain status quo on removal of SRM**
- **To reflect on establishing a limited list of SRM applicable in MS with a negligible risk**
- **To reiterate our request to OIE to work on atypical BSE**

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TSE Road map 2: eradication measures in small ruminants

- **<u>Strategic goal</u>**: to **adapt the current eradication measures** in TSE infected flocks of sheep and goats to bring them in line with the latest scientific knowledge and to develop sustainable tools to control TSE in small ruminant flocks in the EU

- Herd certification

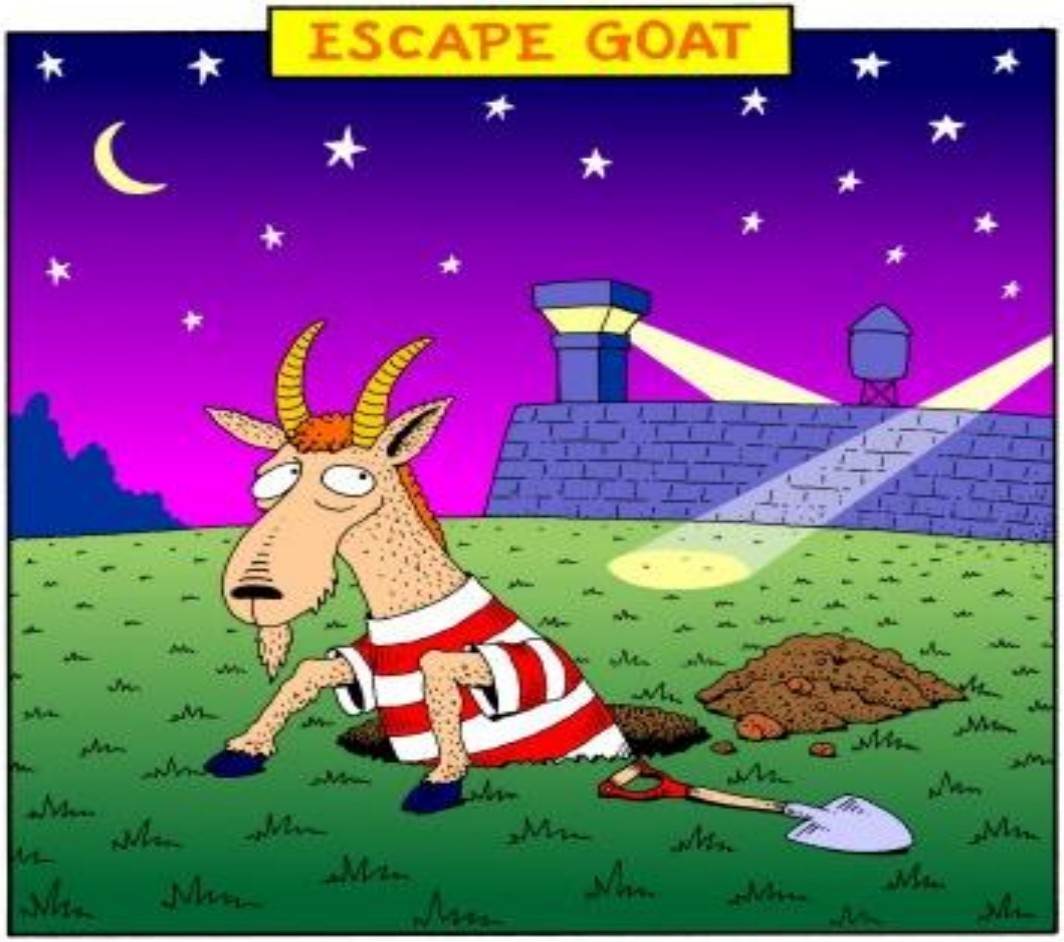
- Measures for Atypical Scrapie

- Genetic resistance in goats

TSE Road map 2: challenges

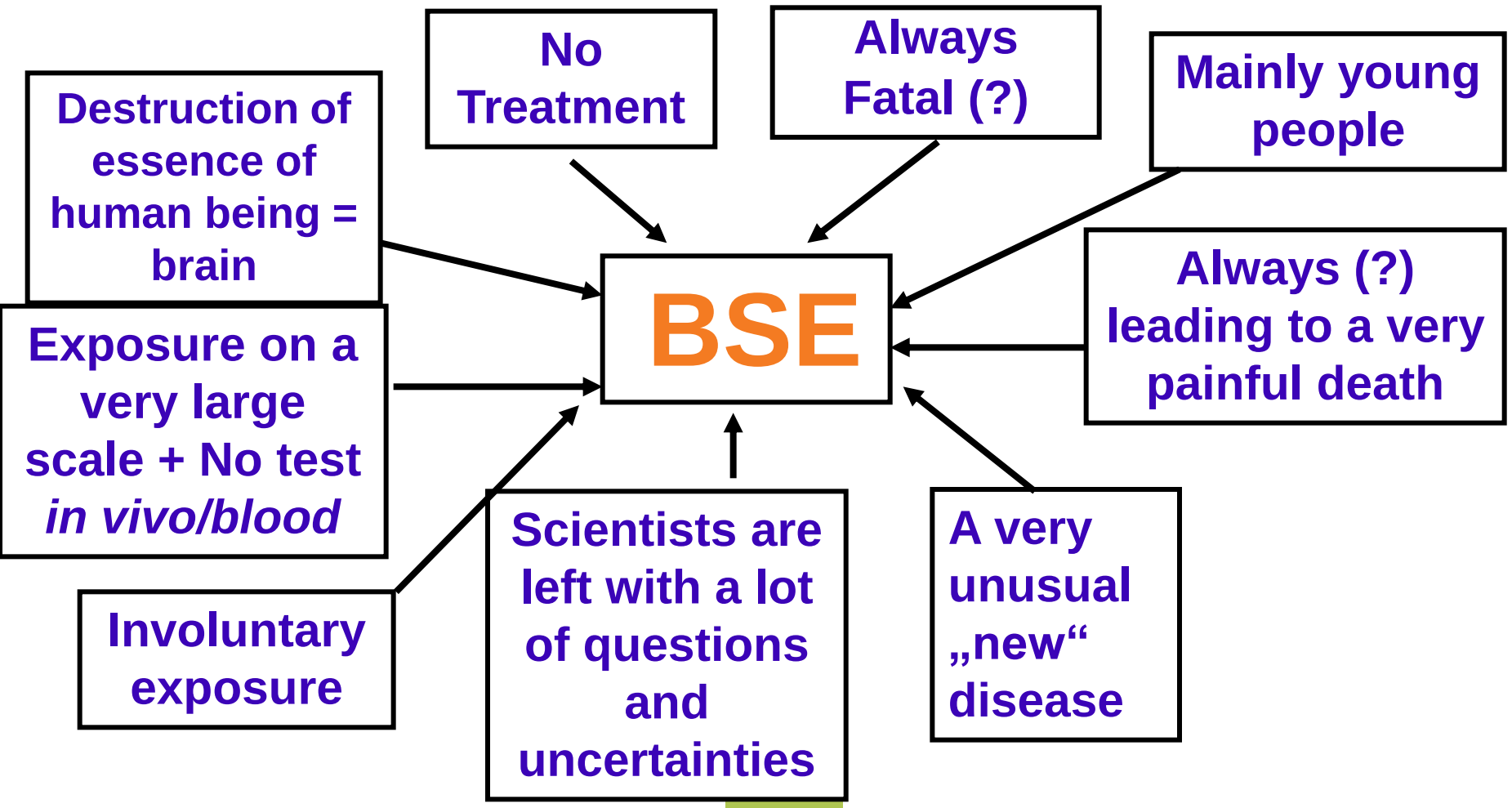
- **Complete elimination of the risk: unrealistic**
  → Proportionality of the measures
- **No complacency**
- **Solid scientific advice: semi-quantitative or quantitative risk assessments taken into account epidemiological situation**
- **Communication towards the consumers**

BSE crisis was one of the reasons for the establishment of "Risk Assessment bodies" such as _EFSA and National Food Safety Agencies_



And vigilance remains

BSE: REASONS FOR CONCERN AMONG THE PUBLIC



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Thank you for your attention

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Time for discussion……

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