REPORT OF THE OIE/FAO-APHCA/DLD
REGIONAL WORKSHOP ON BSE DIAGNOSIS AND SURVEILLANCE

Bangkok, Thailand

19-22 November 2001

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OIE REPRESENTATION FOR ASIA AND THE PACIFIC, TOKYO, JAPAN

AND

ANIMAL PRODUCTION AND HEALTH COMMISSION FOR ASIA AND THE PACIFIC
C/O FAO-RAP, BANGKOK, THAILAND
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The Regional Workshop on BSE Diagnosis and Surveillance  
(Bangkok, Thailand, 19-22 November 2001)

Opening Ceremony

The opening ceremony was held at the Maruay Garden Hotel, Bangkok, Thailand on 19 November 2001. The meeting was attended by about 250 participants and observers from 8 countries/territories; i.e., Hong Kong-China, Taipei-China, Republic of Korea, Philippines, Thailand, Malaysia, Vietnam, and Switzerland (speakers) as well as representative from two international organizations - OIE and FAO.

The opening addresses were made to welcome all of the participants and observers by the following officials; Dr Wallapa Nunbhakdi, Director of National Institute of Animal Health of Thailand, representing the host institute of this workshop on BSE Diagnosis and Surveillance; Dr. Vishnu Songkitti, representing the FAO-APHCA; Dr. Teruhide Fujita, OIE Regional Representative for Asia and the Pacific; and Dr. Prachak Thiratinrat, Assistant Director General, Department of Livestock Development, Thai Ministry of Agriculture and Cooperatives, representing the host country.

Lecturers on BSE

(1) Dr. Dagmar Heim, Swiss Federal Veterinary Office, presented her paper on the overall aspects on Bovine Spongiform Encephalopathy (BSE) Diagnosis and Surveillance. She touched on a wide range of BSE matters including TSE in general, current situation of BSE worldwide, quoting BSE in UK, Switzerland and other countries, epidemiological aspects including age of the BSE cases, cases per herd, surveillance both in a passive and positive way, diagnosis of BSE, targeted surveillance, organization and initial problems of the targeted surveillance in Switzerland, measures taken for animal health and public health, processing of animal waste, effect of the measures concerning feed, elimination of BSE-cases, risk assessment for BSE, problems and lessons to be leaned. (Appendix I)

(2) Dr. David E. Ward, Animal Production and Health Division, Food and Agriculture Organization (FAO) Headquarters, Italy, gave lectures on Bovine Spongiform Encephalopathy, Risk Management Under Different Livestock Production Systems-Veterinary Services’ Capacity and Structures. His paper included themes of how to identify BSE or Transmissible Spongiform Encephalopathy (TSE) cases in a country, effective communication and incentive-based reporting from livestock industry, risk-based, clinical syndrome-based surveillance to identify TSE cases and collaboration with human health authorities. (Appendix II)

(3) Dr. Catherine Botteron, University of Bern, Switzerland presented her paper on sample collection and biosafety, pathogenesis and diagnosis of TSE which included characteristics of TSE, causes of Spongiform Encephalopathy, prion protein, support of the prion theory, BSE pathogenesis, vertical transmission of BSE, species barrier, BSE in transgenic mice, genetic code, neuropathology of TSE, neuronal vacuolation, neuronal degeneration, gliosis, PrP accumulation and differential diagnosis for BSE. (Annex III)
(4) Prof. Andreas Zurbriggen, University of Bern, Switzerland gave lectures on laboratory diagnosis of TSE. His paper covered methods of TSE diagnosis, clinical symptoms including typical and atypical presentation in clinical signs of BSE, factors influencing the number of reported clinical BSE cases, some anti-PrP antibodies, EU-validated BSE rapid tests, required conditions, new attempts to demonstrate PrP, and further tests for TSE, particularly BSE. (Annex IV)

Lectures and Laboratory Practices on BSE Diagnosis and Surveillance

On 20 November 2001, the demonstration of sample collection was made by the lecturers. All the participants practiced this on the prepared cattle heads and became accustomed to taking medulla oblongata out from the cattle head.

Sample preparation for histopathology and IHC followed the practices mentioned above. An emphasis was put on how to separate the brain and to keep the proper portion (in particular obex) by cutting the brain for histopathological and IHC examinations.

The participants were engaged in microscopic observations on the histopathological samples prepared. Those stained brain samples included different types of diseases which provided the participants with the interesting experiences to observe histopathological changes in the brain caused by different diseases including listeriosis, sepsis, brain abscess, lymphosarcoma, ependymoma and brain edema as well as BSE and Scrapie.

The participants were explained by the lecturers about the important and critical points of brain changes caused by BSE and Scrapie on the HE stained and Immunohistochemistry (IHC) sections. Discussions were made on the pathological changes by these diseases.

On 21 November 2001, all the participants joined the course of IHC practices, including Bovine PrP staining on paraffin sections. During the on-going process of preparatory stage of section staining, the lecture using audiovisual aids helped the participants discuss and understand the whole process of preparation for the IHC test.

On 22 November 2001, all the participants were given instructions for preparation of the samples for the rapid tests. They gained theoretical and practical experiences on BSE diagnosis using the ELISA technique. The practices on ELISA test, using negative and positive samples were made in line with the protocol.

In addition, the participants observed both the negative and the positive sections prepared for the IHC test and also those sections stained by the participants, themselves. This gave them good practice on comparison as for real positive and false positive sections as well as the negative ones.

The theory of Western blot was given intensively by the lecturer as well.
Programme of
OIE/FAO-APHCA/DLD
Regional Workshop on BSE Diagnosis and Surveillance
(Bangkok, Thailand, 19-22 November 2001)

Monday 19 November, 2001 (at the Maruay Garden Hotel)

08.00-09.00  Registration

09.00-09.30  Opening Ceremony
• Welcoming Address by Director of National Institute of Animal Health from DLD
• Welcoming Address by Representative from the FAO-APHCA
• Welcoming Address by Representative from the OIE Regional Representation for Asia and the Pacific
• Opening Address by Assistant Director-General from DLD

09.30-10.00  Coffee break

10.00-12.00  Overview BSE Situation
(by Dr. Dagmar Heim)

12.00-13.00  Lunch

13.00-13.45  BSE Risk Management under Different Livestock Production System and Veterinary Services'Capacity and Structure
(by Dr. David E. Ward)

13.45-14.15  Sample Collection and Biosafety, Pathogenesis and Diagnosis of TSE
(by Dr. Catherine Botteron)

14.15-14.45  Coffee break

14.45-15.45  Pathogenesis and Diagnosis of TSE
(by Dr. Catherine Botteron)

15.45-16.30  Laboratory Diagnosis of TSE
(by Dr. Andreas Zurbriggen)
Programme

**Tuesday 20 November, 2001** (at National Institute of Animal Health)

- 09.00-10.00 Sample Collection
- 10.00-10.15 Coffee break
- 10.15-12.00 Sample Preparation for Histology and IHC
- 12.00-13.00 Lunch
- 13.00-14.30 Sample Preparation for Histology and IHC (continued)
- 14.30-14.45 Coffee break
- 14.45-16.30 Microscopic Observation

**Wednesday 21 November, 2001** (at National Institute of Animal Health)

- 09.00-10.00 Immunohistochemistry: Theory
- 10.00-10.15 Coffee break
- 10.15-12.00 Immunohistochemistry: Practice
- 12.00-13.00 Lunch
- 13.00-14.30 Immunohistochemistry: Practice (continued)
- 14.30-14.45 Coffee break
- 14.45-16.30 Immunohistochemistry: Practice (continued)

**Thursday 22 November, 2001** (at National Institute of Animal Health)

- 09.00-10.00 Preparation of the Sample for the Rapid Tests
- 10.00-10.15 Coffee break
- 10.15-12.00 ELISA: Theory and Practice
- 12.00-13.00 Lunch
- 13.00-14.00 ELISA: Practice (continued)
- 14.00-14.15 Coffee break
- 14.15-16.30 Western Blot: Theory
List of Participants

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19 – 22 November 2001, Bangkok, Thailand

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Welcome Address
by
Dr. Wallapa Nunbhakdi
Director of National Institute of Animal Health
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19-22 November 2001

Dr. Prachak Thiratinrat, Assistant Director-General, DLD
Dr. Teruhide Fujita, Regional Representative, the OIE Regional Representation for Asia and the Pacific
Dr. Vishnu Songkitti, APHCA Liaison Officer

BSE Experts
Participants, Observers and Guests
Ladies and Gentlemen

On behalf of the Organizing Committee, the Department of Livestock Development, we are very pleased to host Regional Workshop on Bovine Spongiform Encephalopathy Diagnosis and Surveillance in Thailand. I would like to sincerely thank Dr. Prachak Thiratinrat, Assistant Director-General, Department of Livestock Development for giving us the honor to preside over the opening ceremony this morning. We would like to welcome all participants, experts, observers, guests and the DLD colleagues.

Since BSE has been spread throughout many countries in several parts of the world, not only it is an important zoonotic disease but also it has critical impacts on trade of livestock and their products. This Regional Workshop is then arranged by the cooperation of the Food and Agriculture Organization of the United Nations, the Animal Production and Health Commission for Asia and the Pacific, the Office International des Epizooties and the Department of Livestock Development of Thailand, for objectives on transferring laboratory diagnostic technology, global BSE distribution and disease surveillance.

The Workshop comprises one day of lecture and 3-day of laboratory practice. The lecture will be held today and there will be a total of 200 attendants from DLD, Ministry of Public Health, Municipality, academic institute, and private sectors are welcome to attend. The participants will be 20 for three days of laboratory practice which is only arranged for invited participants due to the available laboratory capabilities of histopathology, immunohistochemistry and ELISA technique. There will be two participants from each of Malaysia, Republic of Korea, the Philippines, Taipei China, Vietnam and one from Hong Kong. The rest are Thai participants from National Institute of Animal Health, Regional Veterinary Research and Diagnostic Centres and Faculty of Veterinary Medicine of Kasetsart University, Chiangmai University, Chulalongkorn University and Khonkean University. Hopefully, it will create awareness, mutual understanding of the disease and networking in BSE surveillance in the very near future. We expected that the Workshop would establish a close cooperation among countries in order to prevent and control the disease within our region.
Opening Address

Ladies and gentlemen, may I request our co-hosts to convey few messages from their organizations on the workshop.

Firstly, Dr. Vishnu Songkitti, APHCA Liaison Officer.

Secondly, Dr. Teruhide Fujita, Regional Representative, the OIE Regional Representation for Asia and the Pacific.

Then, Dr. Prachak Thiratinarat, Assistant Director General, Department of Livestock Development, will declare the “Regional Workshop on BSE Diagnosis and Surveillance” open.

Thank you very much.
Opening address
by
Vishnu Songkitti
FAO-APHCA
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19 November 2001

Dr. Teruhide Fujita, OIE Regional Representative for Asia and the Pacific
Dr. Prachak Tiratinatat, Assistant Director General, Department of Livestock Development
Dr. Wallapa Nunbhakdi, Director of NIAH
Invited experts: Dr. Dagmer Heim, Dr. Catherine Botteron, Dr. Andreas Zurbriggen
My senior FAO colleague, Dr. David Ward,
Distinguished participants, guests, ladies and gentlemen

On behalf of one of the three co-organizers of this Regional Workshop, FAO-APHCA or the FAO Animal Production and Health Commission for Asia and the Pacific is honored to have the opportunity to share with the Office International des Epizooties (the OIE), and the Department of Livestock Development of Thailand in organizing this Workshop.

We have with us this time 20 participants, who are currently working in animal disease diagnostic laboratories from 7 countries in our Asian region, and will stay with us for the whole workshop period of four days. We are glad to be able to accommodate our over 200 guests to listen to the special lectures and presentations on BSE, today.

Also on behalf of Dr. Denis Hoffmann, Secretary of APHCA, who is on duty travel in East Timor this week, I would like to express our gratitude to the team from the Department of Livestock Development who form an organizing committee to work out all local arrangements for us. Special appreciation goes to our experts from Switzerland and from the FAO Headquarters in Rome who, despite their very busy schedules, agreed and come to Thailand to help us with their special presentations today, as well as hands-on training in BSE histopathology and molecular BSE ELISA technique for the next three days. We wish you a very fruitful workshop and a very pleasant stay in Thailand for all our overseas friends.

Last but not least, I am grateful to Dr. Prachak Tiratinatat, Assistant Director General of DLD, for your kind presence here as our Chief Guest this morning.

Thank you so much.
Opening Address
by
Dr. Teruhide Fujita
OIE Regional Representative for Asia and the Pacific
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19-22 November 2001

Dr. Prachak Thiratinrat, Assistant Director General of Department of Livestock Development
Dr. Vishnu Songkitti, FAO/APHCA
Dr. Wallapa Nunbhakdi, Director of National Institute of Animal Health

Distinguished Participants
Ladies and Gentlemen

I would like to welcome all of you to this important workshop on behalf of the OIE Regional Representation for Asia and the Pacific and as one of the organizers of this workshop.

On this occasion, I thank the experts from Switzerland, namely Dr. Dagmar Heim, Dr. Andreas Zurbriggen and Dr. Catherine Botteron and also Dr. David Ward from FAO, Headquarters in Rome, Italy for their kind acceptance to attend our joint workshop as the lecturers.

The same thankfulness should be addressed to the Thai Ministry of Agriculture and Cooperatives for their generosity to host this workshop, and in particular to Department of Livestock Development that made efforts to prepare for the workshop.

As you know well, BSE is the disease recognized firstly in the United Kingdom in 1986 and had generally been considered as a rather specific disease occurred in European countries.

However, taking into consideration the importance of the disease nature to possibly be transmitted from cattle to humans and the fact of importation of meat and bone meal and live cattle from UK and other European countries into other countries in this region, we, the organizers of this workshop including OIE, FAO and Thai DLD considered that awareness and diagnosis/surveillance technologies of the disease should be taken in mind and strengthened even in the Asian and Pacific Region.

The disease was reported in Japan last September, only two months ago, and this was the first case in this Region.

Only one positive case of BSE has really caused the serious socio-economic confusion in the country.

Now, under these conditions, this workshop will provide the participants with the important information and technologies necessary for the preparedness, diagnosis and surveillance of the disease.

We will be able to learn about and to discuss BSE matters through the two major courses, namely the lectures being held today and the individual course for laboratory diagnosis and surveillance to begin at the National Institute of Animal Health, DLD from tomorrow for three days.
Opening Address

I do hope all the participants can take an advantage of this workshop and will become the core persons for the purposes of BSE prevention and control including the setting up of diagnostic and surveillance systems of BSE in their respective countries/territories.

Thank you for your attention.
Opening Address
by
Dr. Prachak Thiratinrat
Assistant Director General, DLD
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19-22 November 2001

Dr. Teruhide Fujita, Regional Representative, the OIE Regional Representation for Asia and the Pacific
Dr. Vishnu Songkitti, APHCA Liaison Officer
Dr. Wallapa Nunbhakdi, Director of National Institute of Animal Health

BSE Experts
Distinguished Participants, Observers and Guests
Ladies and Gentlemen

It is my pleasure to be here this morning on the opening of the Regional workshop on BSE Diagnosis and Surveillance today.

As we all aware, the BSE or Mad Cow Disease has posed threat to human health and economic risk of trade in animals and products of animal origin at both national and international levels. The difficulty in coping with the disease effectively is not only because of diagnostic techniques but a long incubation and no treatment available also. The disease has become a world problem due to its wide distribution since the first occurrence in United Kingdom in 1986. The control and eradication measures need joint regional efforts and enormous resources once it occurs. Therefore, strengthening our cooperation on BSE surveillance may be by all means to protect our region from the disease invasion.

It is so very graceful that the Food and Agriculture Organization of the United Nations, the Animal Production and Health Commission for Asia and the Pacific, and the Office International des Epizooties, in cooperation with the Department of Livestock Development jointly organize this Regional Workshop for the purpose of transferring essential knowledge and modern diagnostic technology on BSE to people in this part of the world. At present, I could say that most of our Asian countries are still innocent for this devastative disease of both in human and livestock. So let me hope that this Workshop would bring about our benefit on regional freedom from BSE in the future. May I now declare the Regional Workshop on BSE Diagnosis and Surveillance open and I wish you all every success.

Thank you.
Report at the Closing Ceremony

by

Dr. Wallapa Nunbhakdi
Director of National Institute of Animal Health
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19-22 November, 2001

Dr. Rapeepong Vongdee, Director General, DLD
Dr. Teruhide Fujita, OIE Regional Representative for Asia and the Pacific
Dr. Vishnu Songkitti, APHCA Liaison Officer

BSE Experts
Ladies and Gentlemen

On behalf of the Workshop Organizing Committee, I would like to thank FAO-APHCA, OIE and DLD for sponsoring this regional workshop on BSE diagnosis and surveillance. The objective of the workshop was distribution of knowledge in global BSE situation, laboratory diagnostic technology and disease surveillance measures to animal health officers in this region. There were approximately 200 persons working in animal health fields attended the BSE lecture session on the first day, but only 20 selected participants from Hong Kong, Republic of Korea, Malaysia, the Philippines, Taipei China, Vietnam and Thailand were allowed to take part in a 3 day laboratory session. Participants were exposed to three diagnostic techniques: histopathology, immunohistochemistry and ELISA. I am convinced that the knowledge and technology the participants has gained from this BSE Workshop will be useful for targeted, active surveillance for BSE in their countries.

I wish all experts and participants have a safe trip back home.

Thank you.
Closing Address
by
Dr. Rapeepong Vongdee
Director General of Department of Livestock Development
Regional Workshop on BSE Diagnosis and Surveillance
Bangkok, Thailand, 19-22 November, 2001

Dr. Teruhide Fujita, Regional Representative, OIE Regional Representation for Asia and the Pacific
Dr. Vishnu Songkitti,APHCA Liaison Officer
Dr. Wallapa Nunbhakdi, Director of National Institute of Animal Health

Esteemed BSE Experts
Distinguished Participants, Observers and Guests
Ladies and Gentlemen

On behalf of the Department of Livestock Development, I am very pleased to learn that the Regional Workshop on BSE Diagnosis and Surveillance has achieved its objectives on transferring the current knowledge and laboratory technology on BSE. I wish you all could exploit what you have learnt during the 4 days workshop at its best in the future work of your country.

One great benefit in the workshop is also to create public awareness on the BSE or Mad Cow Disease through the personnel of concerned authorities in each country. This should start an initial understanding to a variety of questions from the public since the disease has posed threat to human health. Also at the national level, the authorities concerned with both human and animal health can cooperate their activities to conduct an active surveillance in order to protect their own territories including to prevent from a risk of disease being introduced into our region. The long-term benefit will be national and regional economic on trade of animals and products of animal origin. Although to cope with the disease is not easy and very expensive once it occurred, it is a must to do since it is the world’s human and animal health problem and trade discrimination.

We should thank to the FAO-AHPCA and OIE in providing us an opportunity to organize this invaluable and informative workshop to deal with BSE. The Department of Livestock Development is also honored and will render its best cooperation to co-organize such useful workshop like this without any hesitation.

I hope you all are satisfied with what you’ve learnt from this Workshop and enjoy your days in Thailand. Please have a nice journey back home. I wish to see you sometime in the future since we are all working in the animal health field.

Thank you.
Photos of Activities

Opening address by
Dr. Prachak Thiratinrat
Assistant Director General, DLD
(Center)
Dr. Vishnu Songkitti, FAO/APHCA
Liaison Officer (left)
Dr. Teruhide Fujita, OIE Regional
Representation (right)

Participants and speakers on the opening day

IHC slide discussion under multi-channel microscope
Appendix I

Overview BSE Situation
Overview BSE Situation

Slide 1

BSE Diagnosis and Surveillance

OIE/FAO-APHCA/DLD Workshop
19-22 November 2001

Slide 2

- TSE general
- Current situation of BSE worldwide
- Surveillance (passive and active)
- Measures
- Human TSE
- Risk assessment for BSE
- Conclusions
Transmissible spongiform encephalopathies (TSE) first reporting

BSE worldwide
**BSE: Basic events**

- 1986 first BSE case in UK diagnosed
- 1989 first imported cases (Falkland and Oman)
- 1989 first native case outside UK (Ireland)
- 1990 first native case on the European continent (Switzerland)

---

**Origin of BSE in UK**

- Scrapie?
  - Large sheep population in relation to cattle
  - High prevalence of scrapie
- „Sporadic BSE“?
- Antelopes?
- Others?
- Insufficient sterilisation of meat and bone meal
- Use of meat and bone meal in cattle feed
Slide 7

BSE cases in UK

Slide 8

Number of BSE cases in the world
Slide 9

First cases of BSE

First occurrence of indigenous BSE cases


United Kingdom Ireland France Belgium Luxembourg Liechtenstein Switzerland

Portugal Sweden Denmark Germany

Ukraine Czech Rep Slovakia Japan Austria Finland

USA

Slide 10

BSE in some countries
Some epidemiological aspects

Age of the BSE-cases

- Most of the cases between 4-6 years
- Youngest found 20 months
- Oldest found 19 years
### Age of the BSE-cases in UK

<table>
<thead>
<tr>
<th>Year of onset</th>
<th>Age youngest case (mnths)</th>
<th>Age oldest case (yrs.mnths)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986</td>
<td>30</td>
<td>6.07</td>
</tr>
<tr>
<td>1987</td>
<td>30</td>
<td>10.00</td>
</tr>
<tr>
<td>1988</td>
<td>24</td>
<td>11.01(2)</td>
</tr>
<tr>
<td>1989</td>
<td>21</td>
<td>5.04</td>
</tr>
<tr>
<td>1990</td>
<td>24(2)</td>
<td>4.00</td>
</tr>
<tr>
<td>1991</td>
<td>33</td>
<td>4.05</td>
</tr>
<tr>
<td>1992</td>
<td>20</td>
<td>16.02</td>
</tr>
<tr>
<td>1993</td>
<td>28</td>
<td>18.10</td>
</tr>
<tr>
<td>1994</td>
<td>30(2)</td>
<td>6.07</td>
</tr>
<tr>
<td>1995</td>
<td>55</td>
<td>5.05</td>
</tr>
<tr>
<td>1996</td>
<td>29</td>
<td>7.02</td>
</tr>
<tr>
<td>1997</td>
<td>31</td>
<td>5.01</td>
</tr>
<tr>
<td>1998</td>
<td>54</td>
<td>6.05</td>
</tr>
<tr>
<td>1999</td>
<td>39(2)</td>
<td>3.10</td>
</tr>
<tr>
<td>2000</td>
<td>42</td>
<td>9.09</td>
</tr>
<tr>
<td>2001</td>
<td>48(2)</td>
<td>6.07</td>
</tr>
</tbody>
</table>

### Age of the BSE cases in Switzerland

![Bar chart showing age distribution of BSE cases in Switzerland]
Slide 15

**Breed/genetic of the BSE cases**

- No breed-predisposition found
- No genetical predisposition found

Slide 16

**BSE-cases per herd**

- In 96.7-99.5% only 1 case per herd
- Secondary cases
  - Portugal: 3 of 605 herds
  - Germany: 4 of 121 herds
  - Spain: 1 of 70 herds
  - Switzerland: 12 of 395 herds
Slide 17

**Horizontal transmission**

- No evidence
  - epidemiologically
  - experimentally

Slide 18

**Vertical transmission**

- Cohort study from UK: vertical transmission cannot be excluded
- Outside UK no BSE-cases in offspring
- embryo-study: no transmission through embryos
- No infectivity in semen, embryos and ova found
Slide 19

Surveillance

Slide 20

Detection of BSE-cases

- Until 1999 based on the reporting of clinically suspect cases
- Compulsory notification

¬ passive surveillance
Typical clinical signs (1)

- Disturbances in behaviour
  - fearfulness
  - aggressiveness
  - tremors
- Disturbances in locomotion
  - stiff
  - ataxia
  - hypermetria

Typical clinical signs (2)

- Disturbances in sensitivity
  - hypersensitivity to
    - touch
    - light
    - noise
- Weight loss and reduced milk yield
SLIDE 23

**Differential-diagnosis**
- Listeriosis
- Viral encephalitis (sporadic bovine encephalitis, borna disease)
- Bacterial encephalitis
- Brain edema
- Tumors
- Cerebrocortical-necrosis (CCN)
- Cerebellar atrophy (Purkinje cells)
- Metabolic diseases
- Others

SLIDE 24

**Factors influencing the number of reported clinical BSE cases**
- Disease awareness
  - Information, education
- Willingness to notify cases
  - Measures
  - Compensation
  - Stigma
- Laboratory competence
The true prevalence?

- Passive system
- Subjective, dependent on several factors ⇒ variable
  ⇒ difficult to interpret and compare between countries
- Surveillance based on clinical signs alone not sufficient

How to be closer to the truth

- Targeted surveillance in risk populations
- Risk assessment
**Slide 27**

**Diagnosis of BSE**

- Histology
  - spongiform changes
  - neuronal vacuolation
  - neuronal degeneration
  - gliosis
- Immunohistochemistry
- SAF
- Rapid tests

**Slide 28**

**Tests for BSE**

- „rapid tests“ (Prionics-Westernblot, Biorad-ELISA, Enfer-ELISA)
- Histology
- Immunohistochemistry
- SAF
Some difficulties

- Average 5 years incubation period
- All tests currently available
  - only for dead animals
  - brain material
  - detection only at the end of the incubation period

BSE-Infection

- Infection of the calf
- Cow with BSE
- Lifetime
- Max. 6 months
- Detection of BSE agent in brainstem possible
- Average incubation time: 4-6 years
- No detection of BSE possible
Targeted Surveillance

Risk populations

- Adult bovines
  - BSE suspect
  - emergency slaughter
  - fallen stock
  - ?
Targeted surveillance in Switzerland since 1999

- All dead/killed cows
- All emergency slaughter cows
- Random sample of slaughter cows

Targeted surveillance

- rapid test
- Confirmation of positives by histology/immunhistochemistry
**Tested cows - Switzerland**

<table>
<thead>
<tr>
<th>Category</th>
<th>Examined positive 1999</th>
<th>Examined positive 2000</th>
<th>Examined positive 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical suspects</td>
<td>77 (25)</td>
<td>136 (17)</td>
<td>141 (9)</td>
</tr>
<tr>
<td>Died and killed (taken stock)</td>
<td>7’176 (16)</td>
<td>7’380 (8)</td>
<td>7’160 (6)</td>
</tr>
<tr>
<td>Emergency slaughter</td>
<td>3’578 (6)</td>
<td>5’208 (6)</td>
<td>6’198 (6)</td>
</tr>
<tr>
<td>Normal slaughter</td>
<td>7’138 (3)</td>
<td>7’866 (2)</td>
<td>4’604 (1)</td>
</tr>
<tr>
<td>Voluntary normal slaughter</td>
<td>859 (6)</td>
<td>2’226 (2)</td>
<td>109’620 (9)</td>
</tr>
<tr>
<td>Total</td>
<td>18’808 (50)</td>
<td>25’784 (33)</td>
<td>127’923 (30)</td>
</tr>
</tbody>
</table>

**Number of BSE-cases according to year of diagnosis**

<table>
<thead>
<tr>
<th>Year of diagnosis</th>
<th>no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>0</td>
</tr>
<tr>
<td>1991</td>
<td>10</td>
</tr>
<tr>
<td>1992</td>
<td>2</td>
</tr>
<tr>
<td>1993</td>
<td>7</td>
</tr>
<tr>
<td>1994</td>
<td>17</td>
</tr>
<tr>
<td>1995</td>
<td>23</td>
</tr>
<tr>
<td>1996</td>
<td>21</td>
</tr>
<tr>
<td>1997</td>
<td>17</td>
</tr>
<tr>
<td>1998</td>
<td>6</td>
</tr>
<tr>
<td>1999</td>
<td>4</td>
</tr>
<tr>
<td>2000</td>
<td>8</td>
</tr>
<tr>
<td>2001</td>
<td>16</td>
</tr>
</tbody>
</table>

- Feed ban
- Before feed ban
- Born after 12/90
- Born before 12/90
Slide 37

**Targeted surveillance results**

<table>
<thead>
<tr>
<th>Histology</th>
<th>PrP(WB/IHC)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>negative</td>
<td>weak positive</td>
<td>2</td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td>9</td>
</tr>
<tr>
<td>not done</td>
<td>positive</td>
<td>17</td>
</tr>
<tr>
<td>positive</td>
<td>positive</td>
<td>28</td>
</tr>
</tbody>
</table>

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**Surveillance/disease awareness**

- First step:
  - passive surveillance together with good compensation, reasonable measures and active communication
  - active surveillance
    - + cases in active surveillance
    - + cases in passive surveillance
      - disease awareness
      - "no way out"
Active surveillance
-clinical signs-

- 1/3 typical
- 1/3 weak typical
- 1/3 no typical signs of BSE, but other symptoms
  - reduced milk yield and wasting
  - claw problems
  - mastitis
  - recumbency

Surveillance/disease awareness

- Second step:
  - passive + active surveillance
  - strengthened measures for disease awareness
  - punishment
  - intensive ante mortem inspection for cows
    - cases in active surveillance
    + cases in passive surveillance (before slaughterhouse!)
Slide 41

**Number of clinical suspect cases**

![Bar chart showing the number of clinical suspect cases from 1991 to 2002.](chart)

- **Positive** cases
- **Negative** cases

<table>
<thead>
<tr>
<th>Year of Diagnosis</th>
<th>No. of Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>10</td>
</tr>
<tr>
<td>1992</td>
<td>5</td>
</tr>
<tr>
<td>1993</td>
<td>2</td>
</tr>
<tr>
<td>1994</td>
<td>0</td>
</tr>
<tr>
<td>1995</td>
<td>3</td>
</tr>
<tr>
<td>1996</td>
<td>5</td>
</tr>
<tr>
<td>1997</td>
<td>10</td>
</tr>
<tr>
<td>1998</td>
<td>15</td>
</tr>
<tr>
<td>1999</td>
<td>20</td>
</tr>
<tr>
<td>2000</td>
<td>25</td>
</tr>
<tr>
<td>2001</td>
<td>100</td>
</tr>
</tbody>
</table>

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**Introduction of active surveillance in the EU**

- **Spring 2000:**
  North-West-France: fallen stock and emergency slaughter

- **January 2001:** begin of testing in the EU
Organisation and initial problems for the targeted surveillance in Switzerland

Sampling at animal collection points and one central rendering plant

- Veterinarians responsible for
  - sampling
  - marking of samples
  - dispatch to laboratory for rapid tests
Fallen stock

- Traceability
  - marked with special ear tag at collection
  - responsible chauffeurs of transport company or collection centers
  - all accompanied by document

Emergency slaughter

- All vets responsible for emergency slaughter equipped with
  - special spoon
  - instructions
  - container for samples
Regular slaughter

- 7200 samples
- stratified by cantons
- each cantonal veterinarian responsible for the organisation of sampling

Problems

- Samples autolyzed - histology not possible
- no eartag
- no signature of owner ("it was not my cow")
- Late arrival of samples (post) - storing of carcasses
- confusion of samples
MEASURES

- Animal health
  - Direct aim: to eradicate BSE
  - Indirect aim: to reduce human exposure
- Public Health
  - Aim: to reduce human exposure risk
    - food chain
    - others (blood, pharmaceuticals, cosmetics...)

Direct aim: to eradicate BSE
Indirect aim: to reduce human exposure

Aim: to reduce human exposure risk

food chain
others (blood, pharmaceuticals, cosmetics...)
Overview BSE Situation

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**Animal Health**
- feed
- culling
- import

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**Import**
- countries
  - from UK
  - from BSE „affected countries“ (GBR III, unknown status)
- material
  - feed (MBM, BM, concentrates...)
  - live cattle
  - offal
  - others (contamination)
SRM ban in feed

BSE in cattle: infectious tissue
- Brain
- spinal cord
- eye
- trigeminal ganglia
- dorsal root ganglia
- ileum
⇒ safe raw material

Processing of animal waste

133°C/3 bar/20 minutes
Feed ban

- Feed ban of MBM for ruminants
- Measures to avoid cross contamination
  - Separated feed lines
  - General feed ban

Most important measures concerning feed

<table>
<thead>
<tr>
<th>Measure</th>
<th>UK</th>
<th>CH</th>
<th>EU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed ban for ruminants</td>
<td>1988</td>
<td>1990</td>
<td>1994</td>
</tr>
<tr>
<td>SRM ban for feed</td>
<td>1990</td>
<td>1996</td>
<td>2000</td>
</tr>
<tr>
<td>Total feed ban</td>
<td>1996</td>
<td>2001</td>
<td>2001</td>
</tr>
</tbody>
</table>
Effect of the measures concerning feed

UK: Year of birth of BSE cases

- Feed ban for ruminants
- SRM ban for feed
- Total feed ban
Overview BSE Situation

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**Switzerland: Year of birth of BSE cases**

- Feed ban for ruminants
- SRM ban for feed
- 133/3/20 all animal waste
- Total feed ban

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**France: Year of birth of BSE cases**

- Feed ban for cattle
- SRM ban for feed
- Total feed ban
Elimination of BSE-cases

- Passive and active surveillance
- Offspring cull ?
- Herd/cohort cull ?

Offspring cull

- Vertical transmission ?
Herd/cohort cull

- all cattle on case-farm
- all cattle on original farm
- all cattle on case-farm and original farm
- all susceptible animals on the farm
- „feed-cohort“
- „birth-cohort“ (born 1 year before and after the BSE animal and born and raised on the same farm)

Herd/cohort-culling

- Feed cohort would be the ideal approach
- All secondary animals found up to now born within one year before or after the birth on the original farm of the index case
- birth cohort culling the most efficient and motivating approach
Culling in Switzerland

- Until December 1996
  - BSE-case
- December 1996 - June 1999
  - herd-culling (case and original farm)
- since July 1999
  - „cohort“-culling

Definition of birth cohort

- BSE-animal
  - born
- Herd of origin of the BSE animal
  - 1.7.89 sold
  - 1.7.90 dead
  - 1.7.91
Minimising risks for human health

Most important measures concerning food (I)

- Incineration of BSE-cases
- ante mortem inspection
- ban on specified risk material
- ban on mechanically recovered meat
Slide 69

**Most important measures concerning food (II)**

- Ban on specified risk material
- Ban on mechanically recovered meat

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**BSE in cattle: infectious tissue**

- Brain
- Spinal cord
- Eye
- Trigeminal ganglia
- Dorsal root ganglia
- Ileum
**Specified risk material**

<table>
<thead>
<tr>
<th>Specified Risk Material</th>
<th>EU</th>
<th>UK and Portugal</th>
<th>Switzerland</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skull inclusive brain and eyes</td>
<td>&gt;12 mths</td>
<td>&gt;6 mths</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Tonsils</td>
<td>&gt;12 mths</td>
<td>&gt;6 mths</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>&gt;12 mths</td>
<td>&gt;6 mths</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Vertebrae column (spinal ganglia)</td>
<td>&gt;12 mths</td>
<td>&gt;12 mths</td>
<td>cows</td>
</tr>
<tr>
<td>Intestines (ileum)</td>
<td>every age</td>
<td>every age</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Spleen</td>
<td>no</td>
<td>&gt;6 mths</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Thymus</td>
<td>no</td>
<td>&gt;6 mths</td>
<td>&gt;6 mths</td>
</tr>
<tr>
<td>Visible lymph and nerve tissue</td>
<td>no</td>
<td>no</td>
<td>every age</td>
</tr>
</tbody>
</table>

**Most important measures concerning food (III)**

- Testing of normal slaughter cattle over 30 months???
- „a measure to enhance consumer confidence“
- depending on
  - magnitude and stage of epidemic
  - implementation of measures
  - detection capacity before slaughter (disease awareness)
Effectiveness of measures

Intensive control of the implementation !!!

Human TSE
Slide 75

Human TSE

- Creuzfeldt-Jacob-disease (CJD)
  - classical form
  - new form (Variant-CJD)
- Kuru
- Gerstmann-Straussler-Scheinker-syndrome
- Fatal familiar Insomnie

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Classical Creuzfeldt-Jacob-disease (CJD)

- Known since 1920
- forms:
  - 15% familiar or iatrogenic
  - 85% sporadic
- incidence of sporadic cases worldwide similar (1 case per 1 million inhabitants per year)
Variant CJD

- First reported March 1996
- Patients very young (average: 29 years)
- Disease duration longer than CJD: approx. 6 months, V-CJD: approx. 22 months
- Kuru-similar prion-protein-plaques

CJD cases in the UK

<table>
<thead>
<tr>
<th>Year</th>
<th>Sporadic</th>
<th>Iatrogenic</th>
<th>Familial</th>
<th>GSS</th>
<th>vCJD confirmed</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1990</td>
<td>28</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
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<tr>
<td>1991</td>
<td>32</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>36</td>
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<tr>
<td>1992</td>
<td>44</td>
<td>2</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>52</td>
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<tr>
<td>1993</td>
<td>37</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
<td>1994</td>
<td>51</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>59</td>
</tr>
<tr>
<td>1995</td>
<td>35</td>
<td>4</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td>1996</td>
<td>40</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>1997</td>
<td>59</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>10</td>
<td>80</td>
</tr>
<tr>
<td>1998</td>
<td>63</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>18</td>
<td>89</td>
</tr>
<tr>
<td>1999</td>
<td>61</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>15</td>
<td>84</td>
</tr>
<tr>
<td>2000</td>
<td>48</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>28</td>
<td>80</td>
</tr>
<tr>
<td>2001</td>
<td>56</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>20</td>
<td>77</td>
</tr>
<tr>
<td>2002*</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Total</td>
<td>557</td>
<td>40</td>
<td>34</td>
<td>18</td>
<td>113</td>
<td>762</td>
</tr>
</tbody>
</table>
Overview BSE Situation

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**Variant CJD**
- All patients Met/Met Condon 129
- No special consumption pattern
- Some clusters
- No other common factors found

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**Link V-CJD / BSE ?**
- UK since 1996
- Intracerebral transmission of BSE in monkey: florid plaques
- Similar physical-chemical structure of BSE and V-CJD (glycotyping)
- Strain-typing similar
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Risk assessment for BSE

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Risk assessment

Recommendation OIE:
for determination of the BSE status of a country, risk assessment has to be done
- Scientific Steering Committee (EU)
  - risk assessment on the basis of the recommendations of the OIE: Geographical BSE risk ("GBR")
- Others
  - in process
Geographical BSE risk („GBR“)

**Definition**
Qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, at a given point in time, in a country.

**Assumptions**
- Transmission of BSE only through feed
- No „home-made“ BSE (Scrapie and other TSE not taken into account)
- Imports of MBM/cattle from affected countries necessary
Slide 85

**Stability and challenge**

*external challenge*

stability:
- identification and elimination of infected animals
- ability to avoid BSE-recycling

---

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**Challenge**

- import of MBM from UK
- import of live cattle from UK
- import of MBM from BSE affected countries
- import of live cattle from BSE affected countries
Overview BSE Situation

Slide 87

**Stability**

- Ability to identify and eliminate infected animals before they are processed
  - Surveillance
- Ability to avoid BSE recycling through the feed chain
  - Parameters to process animal waste
  - Use of MBM; feed ban; cross contamination
  - Ban on specified risk material (SRM)

Slide 88

**Categories (GBR)**

<table>
<thead>
<tr>
<th>GBR level</th>
<th>Risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Highly unlikely</td>
</tr>
<tr>
<td>II</td>
<td>Unlikely but not excluded</td>
</tr>
<tr>
<td>III</td>
<td>Likely but not confirmed or confirmed at a lower level</td>
</tr>
<tr>
<td>IV</td>
<td>Confirmed at a higher level</td>
</tr>
</tbody>
</table>
**Country categories (GBR)**

<table>
<thead>
<tr>
<th>GBR level</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Argentina, Australia, Botswana, Brazil, Chile, Namibia, New Zealand, Nicaragua, Norway, Paraguay, Singapore, Swaziland, Uruguay, El Salvador, Panama, Costa Rica</td>
</tr>
<tr>
<td>II</td>
<td>Colombia, India, Kenya, Mauritius, Nigeria, Pakistan, Sweden, Canada, USA</td>
</tr>
<tr>
<td>III</td>
<td>Albania, Belgium, Denmark, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Poland, The Netherlands, Romania</td>
</tr>
<tr>
<td>IV</td>
<td>Slovak Republic, Spain, Switzerland</td>
</tr>
<tr>
<td>IV</td>
<td>United Kingdom, Portugal</td>
</tr>
</tbody>
</table>

**Problem:**

What to do with countries not assessed?
Lesson to be learned
What was wrong in Europe?

- No sufficient surveillance
- Risk assessment and subsequently risk management inappropriate
- No risk communication
  - Surprise
  - Consumer confidence destroyed

What is next?

- 1986: only UK-problem
- 1990: problem of some countries
- 2000: problem of Europe
- 200x: problem in other continents!

Conclusion of the Joint WHO/FAO/OIE-meeting June 2001:

„potentially infected material was distributed all over the world”
Trade of MBM/cattle
example

- Switzerland
  1 case: 1990
  Detection and elimination
  internal recycling
  reduced Import restrictions
  90: feed ban
  93: 133/3/20
  96: SRM ban

- European country
  1 case: 2000
  No detection and elimination
  Internal recycling
  90: feed ban
  96: 133/3/20
  00: SRM ban

- Country other continent
  Unknown status

---

Europe - Asia

- 1986: First case reported in UK
- 1990: First native cases on the European continent
- 1994: feed ban EU
- 2000: SRM ban
- 2001: targeted surveillance

- 2001: First case reported in Asia

- ?

- ?

- ?
Summary

- Basic: objective risk assessment
- Detection of the real incidence only possible by targeted surveillance in risk populations
- Aim should be to detect animals before slaughtering
- Strict controls of the implementation of all measures
- Preventive measures already before the first case should be considered
- Learn from the mistakes of the others and do not repeat them
Appendix II

BSE Risk Management under Different Livestock Production Systems and Veterinary Services' Capacity and Structures
Slide 1

Bovine Spongiform Encephalopathy
Risk Management Under Different Livestock Production Systems:

Veterinary Services’ Capacity and Structures

David Ward
Animal Production and Health Division
Food & Agriculture Organization of the United Nations, Rome

Slide 2

![Diagram showing percentage of various symptoms]

- Teeth grinding
- Tremors
- Difficulty in rising
- Difficult to handle
- Hyperaesthesia
- Falling
- Apprehension
- Abnormal behaviour
- Nervous at entrances
- Reduced milk yield
- Temperament change
- Loss of condition
- Locomotor difficulty
- Loss of weight
- Kicking
- Nervousness

PERCENTAGE

0 5 10 15 20 25 30 35 40
Slide 3

ABNORMAL MENTAL STATUS SIGNS
Teeth grinding
Abnormal behaviour
Abnormal ear position
Change in temperament
Apprehension

ABNORMAL SENSATION SIGNS
Head pressing or rubbing
Head shyness
Excessive licking
Hyperaesthesia

CHANGES IN POSTURE & MOVEMENT
Early change in gait
Recumbency
Falling
Abnormal head carriage
Tremors
Ataxia

PERCENTAGE
0 20 40 60 80 100

Slide 4

<table>
<thead>
<tr>
<th>Step</th>
<th>Intensive Systems</th>
<th>Extensive Systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abnormal behaviour – sick or ill animal – first identified</td>
<td>Farmer-Animal Caretakers</td>
<td>Farmer-Animal Caretakers/Animal Health Assistant</td>
</tr>
<tr>
<td>Neurological disease signs recognized</td>
<td>Veterinarian</td>
<td>Animal Health Assistant/Veterinarian</td>
</tr>
<tr>
<td>2. Animal's fate: sale, death, slaughter or render</td>
<td>Abattoir/Renderer</td>
<td>Distress sale to trader or to abattoir</td>
</tr>
<tr>
<td>3. Pre- &amp; postmortem health inspection</td>
<td>National or state regulatory authority at abattoir</td>
<td>National or state regulatory authority at abattoir</td>
</tr>
<tr>
<td>Limited home slaughter</td>
<td></td>
<td>No inspection under local slaughter</td>
</tr>
<tr>
<td>4. Fallen stock</td>
<td>Rendered /limited local disposal</td>
<td>not applicable</td>
</tr>
<tr>
<td>5. CNS tissue sample collection</td>
<td>National/state regulatory authorities</td>
<td>National/state regulatory authorities</td>
</tr>
<tr>
<td>None from home slaughter</td>
<td></td>
<td>None from local slaughter</td>
</tr>
<tr>
<td>6. CNS tissue diagnostic tests</td>
<td>Regulatory authority laboratory or accredited laboratory</td>
<td>Regulatory authority laboratory or reference laboratory</td>
</tr>
</tbody>
</table>
Slide 5

**RISK BASED MANAGEMENT**

- **Import Risk Reduction**
- **Risk Analysis Unit**
  - import risk
  - intl. reporting
  - communication
- **Targeted active clinical syndrome**
- **Risk based commodity-specific import**
- **Inspection and Trace back**
- **Laboratory Diagnosis**
  - histology
  - histochemistry
  - other
- **Disposal of infective material**

Slide 6

**Conclusions --**

- Legislative authority
- Minimal essential infrastructure & capability for targeted, active surveillance
- Balance surveillance costs versus risks
- Reduce disincentives for case reporting
- Positive incentives for case reporting
- Effective risk management units
BSE Risk Management Under Different Livestock Production Systems and Veterinary Services’ Capacity and Structures

Introduction: How to identify bovine spongiform encephalopathy (BSE) or transmissible spongiform encephalopathy (TSE) cases in a country

One goal of national veterinary services is to identify and diagnose all transmissible spongiform encephalopathy (TSE) cases in livestock, pets and zoo animals should they occur in order to eliminate infective material from the food chain and from trade. A high index of suspicion among livestock keepers, pet owners and animal health care providers is essential for identifying suspect TSE cases. Authorities may initially focus on looking for disease in cattle, but need also to target high risk groups of buffalo, if present, hoof stock and pet animals. This paper will assist national authorities to refine surveillance approaches for targeted, active surveillance for TSE diseases.

The first suspicion of any TSE disease could come from reports of abnormal behaviour in livestock, pets or zoo animals. A second “front line” of surveillance could include reports of clinical signs of neurological disease from abattoir inspectors, animal health assistants or technicians, community animal health workers (paravets) or veterinarians. The prominence of one or the other track is country specific. Active, targeted surveillance methodologies are most cost effective for low incidence TSE diseases. Active surveillance is estimated to be 30 times more effective than passive surveillance in detecting BSE cases (OIE).

The first requirement is for a high index of suspicion which recognizes the most frequently reported abnormal behavioural and neurological disease signs (see Figures 1 and 2). The best described TSE is bovine spongiform encephalopathy (BSE) in cattle. BSE is classically described as a neurological disease with a gradual, insidious onset and progression of neurological signs. In the UK, disease progression averages about 2 months before cattle are euthanized on humanitarian grounds but progression may occur over a few weeks to many months (FAO).

Notice (Table 1) that behavioural changes must be detected and reported by livestock caretakers and owners before a definitive diagnosis can be made. The initial behaviour changes (under UK conditions) are subtle and include separation from the herd, reluctance to enter the milking parlor, a change in temperament and apprehension. Veterinarians will not be seeing these first signs - farmers, caretakers or pet owners will. Therefore, national authorities need effective voluntary cooperation from the animal owning public if TSEs are to be even presented for diagnosis. Also note that these signs pertain to modern, developed country husbandry conditions. A farmer who hand milks one cow under a shade tree will not be aware of “reluctance to enter the milking parlor” or “separation from the herd”. There is a need for livestock system specific signs to be reported through astute observation and coupled with confirmatory diagnosis.

Effective communication and incentive-based reporting from livestock industry

Animals with behavioural changes and neurological signs represent the high risk group for TSE diseases. Therefore, national authorities need to obtain samples from this group of suspect
animals in order to confirm or refute the diagnosis of TSE disease. As livestock rearing systems can be quite different from country to country, national authorities need to identify the appropriate people and occupations to target their surveillance campaigns toward. And the ways to reach these people will be country and livestock system specific.

Table 1 indicates the people and occupations potentially involved in disease identification pathways in two different livestock production systems, either of which may predominate in developed or developing countries. Information campaigns, incentives and personal follow-up need to be targeted to people within the most likely pathways which diseased animals follow. Both public authorities and the private sector then need to target the people and critical control points where diagnosis is most likely to be made.

An important difference among countries is recognized at step 2, Table 1. Ill animals in many developing countries are not allowed to die on the farm but rather are sold to local butchers, traders or abattoirs as a distress sale for salvage value. These livestock raisers have a strong “private good” incentive to recover value from animals thought ill or likely to die.

Information messages will have to be tailored to each audience in order to bring about the desired awareness to increase the likelihood of diagnosing TSE cases. As women and children in many countries are primary livestock caretakers, messages need to effectively reach these two groups.

Changing laws and regulations is not an effective short-term way to bring about compliance. Financial incentives for reporting suspicious cases can be offered and disincentives for not reporting must be reduced. Intuitively, one would expect that policies to slaughter single animals or even birth cohorts in a herd would be less of a disincentive to reporting than is whole herd slaughter. Owner assurance of swift and agreed compensation for slaughtered animals would also facilitate reporting. Fallen stock or suspicious cases can be removed from premises at government expense in order to obtain samples for diagnosis and remove potential infective material from the food chain. Incentives and disincentives will likely be country specific.

In the UK, farmer reporting compliance of BSE suspects is very high. Over reporting is evident as approximately 20 percent of slaughtered BSE suspect cattle were found histologically negative. Over reporting, as measured by the percentage of false positive cases, is a measure of quality control for the surveillance system and an indicator of how well the whole system is working.

In the UK, farmers receive 50 percent of market value for culled cattle that are diagnosed as BSE positive. Owners of BSE negative cattle were compensated at 100 percent of market value. Countries adopting whole herd cull policies will need to monitor if the reporting compliance reaches a sufficiently high level to detect all TSE cases.

**Risk-based, clinical syndrome-based surveillance to identify TSE cases**

**High risk animals** in this paper refer to those most likely to have TSE diseases. In the case of BSE, this is not an age group, per se but those animals of sufficient age, at least 24 months, and showing a suggestive syndrome of behavioural or clinical signs. The clinical signs of interest for cattle are described in Figures 1 and 2. Clinical syndrome-based case finding is not a new approach but has proven most successful for the eradication of
smallpox in the 1960s and for markedly reducing poliomyelitis and rinderpest incidence in recent years.

The three high risk categories of livestock (and pets and zoological garden collections) are 1) those that died on the farm (fallen stock), 2) sick animals traded or slaughtered in distress, particularly “downer cows” and 3) animals with neurological disease signs. Most animals in these three categories are expected to enter the pathways found in Table 1. The goal of active surveillance is to ensure that the highest proportion enters the pathway where diagnosis can be made.

Additional risk factors for BSE, under UK animal husbandry conditions, were cattle born into larger herds (more purchased feed with more meat and bone meal) and dairy cattle herds which have higher infection rates than do beef suckler herds. Larger zoological collections may also have purchased more commercial feed containing meat and bone meal. Some species or livestock systems may use little, if any purchased feeds, e.g. buffalo kept mainly for traction, extensive cattle, sheep or goat rearing or livestock which never receive any commercial feed.

An effective targeted, active disease surveillance (TADS) system must be able to detect high risk animals at all four steps depicted in Table 1. National veterinary services need the “soft structures” of effective public communication and positive incentives for the targeted, active surveillance system to function properly. The “hard infrastructures” required include the trained personnel in abattoirs, sales markets and decentralized offices having a disease investigation mandate. It goes without saying that regulatory personnel need sufficient means of transportation and operating budgets for disease investigation, communication and contact with the public. In the UK, positive BSE diagnosis was found directly proportional to proximity to a veterinary investigation centre. These latter ingredients are not always available for developing country veterinary services but are recognized as essential. National treasuries need to provide the necessary budgets if authorities are serious about protecting the public from TSE diseases.

Figure 3 depicts the infrastructure and functions of a minimal essential national system for risk management of TSE diseases. In countries where a semblance of such a system is in place, adding on TSE surveillance is expected only to require activities for focusing on these diseases as a priority. Where national veterinary services are weak, providing all the elements for a TSE surveillance system may require substantial resources and training. Alternatively, national authorities may only focus present resources on an effective TADS system. International donor funding may be required to complement national funding in order to establish the required surveillance capacity.

Once a minimal essential animal disease surveillance system is in place, it will serve the nation to diagnose, report and control a variety of diseases. Therefore, an effective TSE active surveillance system may be the lever for surveillance and control of numerous other diseases of livestock and zoonoses in humans.

Private veterinary practitioners provide a second critical component of any targeted, active surveillance for TSE diseases. In some countries, a private veterinary sector does not exist. But if present, its services need to be incorporated into the nation-wide TSE surveillance scheme. Private veterinary service providers will need awareness building and continuing education in order to raise their index of suspicion, for diagnosing neurological signs, taking samples and working with regulatory authorities on disease investigation. Professional
associations should provide refresher training and national authorities can provide equipment for taking samples.

National veterinary authorities need to facilitate history taking, traceback and central nervous system (CNS) tissue sample collection from high risk categories of animals. Communications with farmers, livestock owners, caretakers, especially women and young people, livestock traders, renderers (if any), veterinary health assistants, veterinarians, including their associations, and livestock producer associations are all stakeholders which can be targeted for cooperation. The objective is to keep high risk suspect animals within the surveillance system where they can be sampled and removed from the food or trade chain.

Within a people-oriented framework, the various technical support and logistical systems can be set up by national authorities. For instance, abattoirs and renderers will need large containers (10 litres) and large quantities of buffered formalin for preserving CNS tissues and histological examination. The International Office of Epizootics web site (http://www.oie.net) well describes the protocol for BSE diagnostic confirmation. A questionnaire for collecting information on clinical signs and their duration, differential diagnosis, treatments given and response, age and feeding history, among other signs, would need to take specific livestock rearing systems into account. An animal traceback system would be most useful for locating the origin and their birth cohorts of sick animals and fallen stock. Clearly, traceback should not be a disincentive for owners to report cases.

Collaboration with human health authorities

Human health authorities need to undertake surveillance for TSE cases, in particular variant Crutzfeld-Jakob disease. Veterinary authorities can collaborate closely with the view to follow up human cases to traceback the farm, pet or livestock contacts. Clearly, the occurrence of human TSE disease should alert authorities to the possibility that transmission from TSE infected indigenous animals could have occurred. This, however, is only one of several possible sources of infection in humans and an in-depth investigation is warranted into each human case. An investigation into each human case needs to identify the epidemiology and risk factors involved.

Conclusions

Clearly, countries need legislation to make BSE and other TSE diseases notifiable to national authorities. Legal authority to examine or seize suspect animals is required as is the obligation, and sufficient funds, to pay equitable compensation.

Disincentives to report suspicious TSE cases must be replaced with clear incentives for livestock owners to report. Authorities need to carefully evaluate the potential reporting disincentive of whole herd culling when one TSE positive animal is detected verses a limited culling of only positive animals and their birth cohorts. The International Office of Epizootics code classification of free or infected countries or zones also needs to encourage active disease surveillance and reporting.

Useful incentives for reporting in various developed countries include: coverage of costs to remove and destroy animals dying on farms, zoological parks and homes; owners of culled suspect cases compensated at fair market value for their losses; and diagnosis and
compensation payments made promptly. Such incentives likely apply in Asian countries but others need to be sought as well.

A minimal essential infrastructure and capability for targeted, active surveillance is required in order to find and diagnose TSE diseases in both animals and humans. The core structures are relatively light in terms of infrastructure. Trained human resources and abundant contact with the public are essential. Public awareness messages and surveillance systems will have to be tailored to various livestock production systems and will vary among countries. There is an urgent need for developing countries to report the behavioural abnormalities and neurological signs observed in positive BSE cases, if and when they occur.

Care needs to be taken that the costs of a TSE surveillance system are commensurate with the risk of the diseases being either imported or propagated within a country or already present in indigenous animals. The capacity for risk analysis is essential to obtain information, not only on TSE disease status, but also on matching the surveillance costs with the risk.

An effective risk analysis unit is essential in order to manage the risks from imports, for reporting on TSE status and for communicating health risks to the public. Likewise, such a unit would liaise with decision makers in all aspects of TSE risk management and communication.
Figure 1. Frequencies of the initial clinical signs reported for histologically confirmed cases of BSE in the UK (adapted from the Manual on Bovine Spongiform Encephalopathy, FAO, 1998)

Teeth grinding

Tremors

Difficulty in rising

Difficult to handle

Hyphaesthesia

Falling

Apprehension

Abnormal behaviour

Nervous at entrances

Reduced milk yield

Temperament change

Loss of condition

Locomotor difficulty

Loss of weight

Kicking

Nervousness
Figure 2. Frequencies of abnormal behavioural and neurological signs in BSE positive cattle in the UK (adapted from Manual on Bovine Spongiform Encephalopathy, FAO, 1998)

<table>
<thead>
<tr>
<th>ABNORMAL MENTAL STATUS SIGNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teeth grinding</td>
</tr>
<tr>
<td>Abnormal behaviour</td>
</tr>
<tr>
<td>Abnormal ear position</td>
</tr>
<tr>
<td>Change in temperament</td>
</tr>
<tr>
<td>Apprehension</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ABNORMAL SENSATION SIGNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Head pressing or rubbing</td>
</tr>
<tr>
<td>Head shyness</td>
</tr>
<tr>
<td>Excessive licking</td>
</tr>
<tr>
<td>Hyperesthesia</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>CHANGES IN POSTURE &amp; MOVEMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early change in gait</td>
</tr>
<tr>
<td>Recumbency</td>
</tr>
<tr>
<td>Falling</td>
</tr>
<tr>
<td>Abnormal head carriage</td>
</tr>
<tr>
<td>Tremors</td>
</tr>
<tr>
<td>Ataxia</td>
</tr>
</tbody>
</table>

PERCENTAGE 0  20  40  60  80  100
Figure 3. Minimal essential national veterinary service structure for risk management of BSE / TSE diseases

**Risk Based Management**

**Import Risk Reduction**
- Export country evaluation/inspection (confidence building)

**Risk Analysis Unit**
- Import risk
- Intl. reporting
- Communication

**Risk-based Surveillance**
- Targeted, active clinical syndrome-based case finding in high risk groups

**Inspection and Traceback**

**Laboratory Diagnosis**
- Histology
- Histochemistry
- Other

**Disposal of Infective Material**
Table 1. Critical control points for targeted, active surveillance for BSE / TSE diseases in livestock, zoological collections or the pet population

<table>
<thead>
<tr>
<th>Steps</th>
<th>More intensive, industrial livestock production systems</th>
<th>More extensive, semi-industrial livestock production systems</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abnormal behaviour – sick or ill animal – first identified</td>
<td>Farmer-Animal Caretakers</td>
<td>Farmer-Animal Caretakers / Animal Health Assistant</td>
</tr>
<tr>
<td>Neurological disease signs recognized</td>
<td>Veterinarian</td>
<td>Animal Health Assistant / Veterinarian</td>
</tr>
<tr>
<td>2. Animal’s fate: sale, death, slaughter or render</td>
<td>Abattoir / Renderer</td>
<td>Distress sale to trader or to abattoir</td>
</tr>
<tr>
<td>3. Pre- &amp; post-mortem health inspection</td>
<td>National or state regulatory authority at abattoir</td>
<td>National or state regulatory authority at abattoir</td>
</tr>
<tr>
<td></td>
<td>Limited home slaughter</td>
<td>No inspection under local slaughter</td>
</tr>
<tr>
<td>4. Fallen stock</td>
<td>Rendered / limited local disposal</td>
<td>Not applicable</td>
</tr>
<tr>
<td>CNS tissue sample collection</td>
<td>National / state regulatory authorities</td>
<td>National / state regulatory authorities</td>
</tr>
<tr>
<td></td>
<td>None from home slaughter</td>
<td>None from local slaughter</td>
</tr>
<tr>
<td>CNS tissue diagnostic tests</td>
<td>Regulatory authority laboratory or accredited laboratory</td>
<td>Regulatory authority laboratory or reference laboratory</td>
</tr>
</tbody>
</table>

**Bolding** refers to most likely contact persons or pathway for animals to follow. CNS tissue refers to central nervous system, usually the brain or selected parts (medulla oblongata).

1. Planning awareness building needs to be particularly gender sensitive as women and young people are frequently the main animal caretakers.
Bibliography


Appendix III

Sample Collection and Biosafety, Pathogenesis and Diagnosis of TSE
Sample Collection and Biosafety, Pathogenesis and Diagnosis of TSE

Slide 1

Bovine spongiform encephalopathy

Slide 2

Spongiform encephalopathies

<table>
<thead>
<tr>
<th>Animal</th>
<th>Scrapie</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transmissible mink encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Chronic wasting disease</td>
</tr>
<tr>
<td></td>
<td>Bovine spongiform encephalopathy</td>
</tr>
<tr>
<td></td>
<td>BSE in cat, puma, cheetah, nyala, kudu, oryx, etc</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Man</th>
<th>Kuru</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gerstmann-Sträussler</td>
</tr>
<tr>
<td></td>
<td>Fatal familial insomnia</td>
</tr>
<tr>
<td></td>
<td>Creutzfeldt-Jakob Disease (CJD)</td>
</tr>
<tr>
<td></td>
<td>variant CJD</td>
</tr>
</tbody>
</table>
Causes of spongiform encephalopathies

- Spontaneous: Scrapie, CWD, CJD
- Hereditary: GS, FFI
- Oral transmission: TME, BSE, Kuru

Transmissible spongiform encephalopathies (TSE)

- Transmissible
- Long incubation period
- Progressive and lethal
- Spongiform degeneration of CNS
- Accumulation of amyloid (prion protein)
Prion protein

- The structure of the normal prion protein (PrPc) is known; its function is unknown

- Conversion of PrPc to PrPSc
  
  alpha-helices → beta-pleated sheets
  protease-sensitive → protease-resistant
Slide 7

Contamination → Symptoms

1 year → 5 year

Incubation time

Slide 8

Virus structures

- In the CNS of scrapie-infected hamster
- CNS of sporadic and familial CJD
Support of the prion theory

- PrP mutants in people
- PrP polymorphism in sheep and mice
- Transgenic mice

TSE pathogenesis

Mostly mouse and hamster models

Ingestion → Peyer’s patches → Lymphoid tissues

Nervus vagus → Medulla → Spinal cord → Brain

N. splanchnicus phrenicus
Slide 11

BSE pathogenesis

- Oral infection
- agent in distal ileum 18 months p.i.
- no agent in lymphoid tissues
- how does the agent travel to the CNS?

Slide 12

BSE pathogenesis

- Oral infection of calves
  - sequential killing
  - infectiosity assay (i.c. in mice) of 44 tissues

- ileum, eyes, CNS, spinal and trigeminal ganglia

- No primary replication in lymphoid organs
- direct spread by way of PNS ???
Slide 13

Prions

Slide 14

Vertical transmission of BSE

- Incidence 1% - 10%
- mode of transmission unknown (same environment, same genetics ??)
- no infectiosity in placenta, blood, milk

maternal transmission is not significant
Slide 15

SPECIES BARRIER

Slide 16

What determines the species barrier?

Genetic code for prion protein (PrP)
- Mouse and Hamster have a different PrP code
  = Mice are relatively unsusceptible to hamster prions
Slide 17

BSE in transgenic Mice

- Normal mouse
- Mouse with bovine prion gene
- Mouse with human prion gene

Slide 18

Hamster prion

- Normal mouse
  - Mouse stays healthy
- Mouse with hamster prion gene
  - Mouse dies
Slide 19

Genetic code

- Plays a role in the species barrier
- But is not the only factor
- Other factors (yet unknown) are important as well

Slide 20

Neuropathology of TSE

- spongiform changes
- neuronal vacuolation
- neuronal degeneration
- gliosis
- accumulation of prion protein
Slide 21

Spongiform changes

- mostly grey matter
- bilateral symmetrical
- in predilection areas
- intensity variable
- vacuolation of neuronal processes

Slide 22

Neuronal vacuolation

- in certain predilection areas
- variable size number
- mostly empty
- often no additional neuronal change
- vacuolation can be normal in certain areas!
Neuronal degeneration

- in brainstem nuclei
- chromatolysis
- nuclear changes
- necrosis
- neuronophagia
- neuronal loss

Gliosis

- Astrocytic hyperplasia / proliferation
- associated / not associated with spongiform changes
- mild in BSE, severe in scrapie
- GFAP staining is useful
PrP accumulation

- immunohistochemistry on paraffin sections
- polyclonal / monoclonal antibodies
- specific distribution / appearance
- with or without spongiform changes
- plaques in certain TSE

Differential diagnoses for BSE

- listeriosis
- non-suppurative encephalitis / vasculitis
- cerebrocortical necrosis/
  polioencephalomalacia
- brain edema
- metabolic encephalopathy
- tumors
- others
Appendix IV

Laboratory Diagnosis of TSE
Laboratory Diagnosis of TSE

Slide 1

Slide 2

Methods of TSE Diagnosis

- Clinical Symptoms
- Histology
- PrPSc
  - Immunocytochemistry
  - SAF isolation
  - Western blot (Prionics test)
  - ELISA (Enfer test, Platelia)
- Others in development
- Animal transmission
- Transgenic animals
Slide 3

**BSE Infection & Progression**

- Infection of calf in first year of life
- Incubation
- Detection of PrP\(^{sc}\) possible
- Ca ½ Year before death
- No evidence of BSE

Average age at onset of clinical signs: 65 months (22 months - 18 years)

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Slide 4

**Clinical signs in BSE**

- highly constant lesion profile
- certain signs are frequent

**However:**
- variable clinical presentation
- severity of signs does not correlate with severity of lesions
- many cases are not spectacular
- none of the signs are typical/pathognomonic
Clinical diagnosis of BSE

- Adult animals
  - >3 years
  - average 5 years
- Insidious onset
- Progressive

Typical presentation

- Abnormal behaviour
- Abnormal response to sensory stimuli
- Abnormal motor function
- Loss of condition
Slide 7

**Behavioural signs**

- Nervousness
- Apprehension, fear
- Change of behaviour towards familiar people
- Increased nose licking, head shaking
- Kicking
- Increased vocalisation
- Altered social status in the herd

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**Increased response to stimuli**

- Hyper reactivity to
  - sound,
  - light,
  - touch, esp. head and neck
- Kicking on touching legs
Abnormal motor function

- Abnormal head posture
- Difficulty in rising, recumbency
- Abnormal gait: - spasticity
  - ataxia
  - hypermetria
  - weakness
- Spontaneous muscle activity: - twitching
  - tremor

Extraneural signs

- weight loss
- reduced milk yield
- reduced ruminal contractions
- bradycardia
Slide 11

**Suspicion of BSE**

- diffuse neurological signs
- cerebral signs
- combination of:
  - abnormal behaviour
  - excessive reaction to sensory stimuli
  - ataxia

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**Moral of the story**

**Adequate BSE surveillance**

=  

**Detection of neurologic disease in adult cattle with subsequent post mortem laboratory examination**
Atypical presentation in BSE

Retrospective evaluation:
- 1/3: typical BSE signs
- 1/3: + CNS signs
- 1/3: no CNS signs
  - reduced milk yield
  - weight loss
  - mastitis, foot problems, peritonitis
  - recumbency

Factors influencing the number of reported clinical BSE cases

- Disease awareness
  - information, education

- Willingness to notify cases
  - Stigma
  - Measures
  - Compensation

- Laboratory competence
Slide 15

Some Anti PrP antibodies:
6H4

A monoclonal antibody derived from PrP-null mice, immunized with recombinant PrP of the bovine sequence (Prionics Ltd., Zürich, Switzerland). It binds to SDYEDRYYREN, an epitope in the center of the protease resistant core. Use: Western blot, ELISA, IHC Specificity: bovine, human, pig

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Some Anti PrP antibodies:
Anti Recombinant PrP Serum

A rabbit antiserum, raised against whole length bovine recombinant PrP. Use: Western blot, ELISA, IHC Specificity: bovine, sheep
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**Some Anti PrP antibodies: C15S**

A rabbit antiserum, raised against a peptide of the bovine PrP sequence, GQGTHGQWNKPS, located near the N-terminal of the protease resistant core.

Use: ELISA, IHC

Specificity: bovine

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**Some Anti PrP antibodies: C17V**

A rabbit antiserum, raised against a peptide of the PrP sequence, QRESQAYYQRGASV, located near the C-terminal of the protease resistant core.

Use: ELISA, IHC

Specificity: bovine, sheep
EU-validated BSE rapid Tests

- Western Blot from Prionics AG, Zürich
- ELISAs from Enfer (IRL) and BioRad (F)

- Results comparable to IHC, but results within a few hours and higher quantity possible
- Test also possible with poor sample condition (autolysis) E.g. cadaver

What and why do we test?

- BSE tests allow an epidemiological assessment of the epidemic and enable epidemiological clarifications

- Testsituation:
  - Passive Surveillance
  - Active Surveillance
  - Voluntary BSE Tests

- Laboratories that meet the required conditions, are allowed to test samples for butchers, slaughter houses and private persons
**Required conditions**

1. **Bio safety:**
   - Application at BUWAL
2. **Schooling**
   - Proof of schooling from Test selling company
   - Head of laboratory + deputy
3. **Test**
   - Visit reference centre, checklist, report, round robin test
4. **Tracing of tested animals**
   - Form, defined data, given mask for database

**Ring trial**

- 16 samples are send with express mail to laboratory in the evening
  - 7 samples with homogenise of animal without BSE
  - 1 sample with homogenise of animal with BSE
  - 8 samples with homogenises of mixed dilutions of animals with and without BSE (1:20)

- Results and western blots are sent to the reference centre the next morning via mail
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### BSE-tests since 2001

<table>
<thead>
<tr>
<th>Category</th>
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<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
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<td>Voluntary normal slaughter</td>
<td>109'620</td>
<td>4'804</td>
<td>6'198</td>
<td>7'160</td>
<td>141</td>
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<tr>
<td>Officially ordered normal slaughter</td>
<td>6'198</td>
<td>7'160</td>
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<tr>
<td>Sick slaughter</td>
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<td>Fallen stock</td>
<td></td>
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<tr>
<td>Clinical suspect cases</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| Total tested     | 109'620 | 4'804 | 6'198 | 7'160 | 141   |
| Positive         | 9       | 1     | 8     | 3     | 9     |

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### Active surveillance

- **Focussing on main risk groups:**
  - emergency/ sick slaughter cattle
  - fallen stock (carcasses)
- **Post mortem detection of PrP<sup>Sc</sup> accumulation in brain**
- **Negative test result is no guarantee that an animal is BSE-free (not incubating the disease)**
Why new TSE-Tests?

- Veritable rapid test (minutes instead of hours)
- Animals in incubation (preclinical)
  - Elevated analytical sensitivity
  - Tissues other than CNS (Where resides infectiosity? Where is PrP protein hidden during incubation?)
  - Use of marker different form PrP<sub>Sc</sub>
- Tests in the living animal (ante mortem)
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![Graph showing A*/A values for different samples]

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**New attempts to demonstrate PrP**

- **Eigen/Riesener Test May/June '01**
  - Crosscorrelation-fluorescence spectroscopy:
    - two fluorescent antibodies bind to both PrP<sup>c</sup> and PrP<sup>Sc</sup>;
    - if present, the signal of long PrP<sup>Sc</sup>-aggregates is easily distinguishable from PrP<sup>c</sup> single molecules
  - Measurement of intensity of fluorescence with Laser beam
New attempts to demonstrate PrP

- **Eigen/Riesener Test May/June ’01**
  - Up to now use of brain homogenate and cerebrospinal fluid
  - New Test: 1000fold more sensitive than tests before
  - BUT: Is it possible to detect BSE months earlier than before with this method???
  - Attempts to enhance sensitivity by accumulation of “BSE agent” (elimination of excess PrP, treatment with ultrasound)
  - Not yet ready for routine use, more work necessary to improve test

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New attempts to demonstrate PrP

- **Schmerr/Takahashi (1999-2001)**
  - Probe mixed with defined amount of fluorescent peptide (binds antibody like PrP), addition of defined amount of PrP-specific antibody
  - Free and bound peptide separated by HPLC or capillary electrophoresis, intensities of fluorescence are compared
  - Demonstration of scrapie infection in sheep a few weeks p.i.
New attempts to demonstrate PrP

- Blood test, Boehringer/Ingelheim (2000)
  - Elevated amount of normal PrP as marker for BSE infection in specific peripheral blood cells
  - Alleged “yes-no” test: if animal not infected -> no PrP in specific peripheral blood cells; if animal infected -> always presence of PrP in these cells. BUT: As yet not known whether these cells also PrP positive in other diseases
- Cooperation with ENFER, Ireland
- Actually work in progress to determine at which stage of incubation the test becomes positive
- Publication in press announced; test should be ready for use from 2002

14-3-3-marker in cerebrospinal fluid

- Commercial kit by Californian company
  - Demonstrates specific protein (14-3-3) in cerebrospinal fluid
  - 14-3-3 elevated in most patients with CJD and also in cattle with BSE
  - Allegedly 99% specific and 96% sensitive for CJD
    - (but also positive in 50% of patients with viral encephalitis!)
    - Suitable as additional test for ante mortem confirmation of patients with clinical CJD symptoms (dementia)
- Is not a specific test for BSE (Vet. Rec. 143, 50-51)
Further Tests for TSE, particularly BSE

- At least 10 different companies worldwide in search of a more sensitive and more rapid BSE test (marker or PrPSc)
- So far, none of the “successful” tests announced in the press have been scientifically confirmed as valid
- Does a BSE test performed with extraneural tissue make sense?
- TSE tests – ante mortem and with extraneural (lymphoid) tissues – established and in use for Scrapie, CWD and vCJD

Tonsillectomy for the diagnosis of sheep scrapie

- Developed by ID-DLO (Lelystad, Netherlands)
- Removal of tonsillar tissue, fixation and immunohistochemistry to demonstrate PrPSc
- Recognizes scrapie infection in scrapie susceptible sheep genotypes from about 10-12 weeks p.i
- Amount of PrPSc in tonsils diminishes again towards the end of incubation
Eyelid test for TSE in sheep

- Developed in the USA by USDA Laboratory
  - (O’Rourke et al., 1998, Vet. Rec. 142, 489-491)
- Removal of lymph follicles of the third eyelid under local anesthesia, fixation and immunohistochemistry for the demonstration of PrPSc
- Recognizes sheep scrapie in susceptible sheep genotypes from about 10-12 weeks p.i.