FOOD SAFETY

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Consideration of Risk Variations in Japan Derived from the Proposed Revisions of the Current Countermeasures against BSE

Full Report

Food Safety Commission of Japan*

The Food Safety Commission of Japan (FSCJ) conducted assessments on human health risks associated with the Bovine Spongiform Encephalopathy (BSE) agent, in response to requests from the Ministry of Health, Labour and Welfare of Japan (MHLW). FSCJ conducted risk assessments under the assumption that the present feed control measures are maintained in Japan, the US, Canada, France and the Netherlands. The assessments were also based on the current BSE status and infection risks in cattle in these countries, and interspecies barrier of BSE transmission between cattle and humans. Consequently, FSCJ concludes that in these countries, variant Creutzfeldt-Jakob disease (vCJD) is highly unlikely to develop in association with consumption of BSE prions through meat and offal (excluding the tonsils and distal ileum) from cattle at or under 30 months of age. Conclusions on the domestic and border measures are as follows: 1) Domestic measures: Negligible influences to human health are predicted from the changes in the age limit for BSE testing of cattle in Japan from the current 20 months to 30 months; changes in definition on age of specified risk materials (SRMs: skull excluding tonsils, spinal cord and vertebral column) from “all ages” to “over 30 months of age” in Japan have negligible influences to human health: 2) Border measures: Negligible influences to human health are predicted from the changes in the age restriction from the current 20 months to 30 months on cattle meat and offal imported from the US and Canada; negligible influences to human health are predicted between the current ban and implementation of the age restriction of 30 month on import of cattle meat and offal from France and the Netherlands; changes in definition on age of SRMs (skull excluding tonsils, spinal cord and vertebral column) from “all ages” (equivalent to “import ban” for France and the Netherland) have negligible influences to human health.

Key word: BSE, SRM, BSE testing, domestic measure, border measure

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*This is an English translation based on the original risk assessment report in Japanese (October 2012-FS/931/2012). Only original Japanese texts have legal effect.
The original full report is available in Japanese at http://www.fsc.go.jp/fsciis/evaluationDocument/show/kya20121219037.
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Abbreviations: AFSSA: Agence Française de Sécurité Sanitaire des Aliments, ATQ: Agri-Traçabilité Québec, BARB: born after the reinforced ban, BASE: bovine amyloidotic spongiform encephalopathy, BSE: bovine spongiform encephalopathy, CCIP: Canadian Cattle Identification Program, CFIA: Canadian Food Inspection Agency, CJD: Creutzfeldt-Jakob disease, CMPAF: cattle material prohibited from animal feed, CNS: central nervous system, CPP: continuous Peyer’s patch, DDVS: Directions Départementales des Services Vétérinaires, DGAL: Direction Générale de l’Alimentation, DPP: discrete Peyer’s patch, DRG: dorsal root ganglion, EFSA: European Food Safety Authority, ELISA: enzyme-linked immuno-sorbent assay, EU: European Union, GHP: good hygiene practice, HACCP: hazard analysis and critical control point, H-BSE: H-type bovine spongiform encephalopathy, i.c.: intracerebral, i.p.: intraperitoneal, ID_{50}: 50% infective dose, IHC: immunohistochemistry, L-BSE: L-type bovine spongiform encephalopathy, MAFF: Ministry of Agriculture, Forestry and Fisheries of Japan, MBM: meat-and-bone meal, MHLW: Ministry of Health, Labour and Welfare of Japan, MM: methionine/methionine, mpi: months post-inoculation, MRM: mechanically recovered meat, MV: methionine/valine, NAIS: National Animal Identification System, OIE: Office International des Epizooties, PP: Peyer’s patch, PrP: prion protein, PrP^{Sc}: abnormal prion protein, SBO: specified bovine offal, sCJD: sporadic Creutzfeldt-Jakob disease, SRM: specified risk material, SSOP: sanitary standard operation procedure, TBM: tingible body macrophages, Tg: transgenic, TSE: transmissible spongiform encephalopathy, USDA: United States Department of Agriculture, vCJD: variant Creutzfeldt-Jakob disease, VLA: Veterinary Laboratories Agency, VV: valine/valine, VWA: Nederlandse Voedsel- en Warenautoriteit, WB: western blotting

I. Background and History of the Assessment

1. Introduction

A number of bovine spongiform encephalopathy (BSE) cases were reported mainly in the UK as well as in other European countries, and this epidemic peaked in the early 1990's. According to the relevant reports issued by WHO and others, a possible link between variant Creutzfeldt-Jakob disease (vCJD) and BSE was first reported in 1996. In September 2001, the first BSE case in Japan was identified. Considering the above situation, the Japanese government issued a notice restricting the use of meat-and-bone meal (MBM) containing ruminant tissues for ruminant feed, and implemented various BSE countermeasures comprising of domestic and border measures.

The Food Safety Commission of Japan (FSCJ) conducted self-tasking risk assessments and released the following reports: 1) "Measures against Bovine Spongiform Encephalopathy (BSE) in Japan (Interim Report)"; 2) "Food Safety Risk Assessment Related to Measures against Bovine Spongiform Encephalopathy (BSE) in Japan (May 2005)." and 3) "Risk assessment concerning the comparability between risks of consuming cattle meat and offal regulated by the beef export verification program of the United States/Canada and risks of consuming cattle meat and offal of Japanese cattle (December 2005)." Subsequently, FSCJ conducted a self-tasking risk assessment entitled 4) "A Risk Assessment on Cattle Meat and Offal Imported to Japan (Australia, Mexico, Chile, Costa Rica, Panama, Nicaragua, Brazil, Hungary, New Zealand, Vanuatu, Argentina, Honduras, Norway: from February 2010 to May 2012)."

The Ministry of Health Labour and Welfare of Japan (MHLW) requested FSCJ to conduct risk assessment to be used for revising the present countermeasures against BSE.

2. Background of Requests

When MHLW requested the risk assessment in December 2011, ten years had already passed since the first introduction of BSE countermeasures in Japan. Considering the impacts of the countermeasures and changes of international circumstances, the existing countermeasures on food safety need to be re-evaluated based on the latest scientific knowledge.

The domestic measures have been in place for approximately six years since the last risk assessment was conducted in May 2005. Therefore, reassessment of the measures needs to be based on the latest knowledge including the results of the past BSE surveillance, the impacts of feed control measures enforced in 2001, the results of experimental studies on infectivity of young BSE cattle tissues in mice, and the results of other experimental studies conducted in Japan and other countries.

The border measures for beef imported from the US and Canada have also been in place for approximately six years since the last risk assessment in May 2005, and also the provisional import ban on beef from other BSE countries has been in place for approximately ten years. Recently, the measures have been revised stepwise based on the results of risk assessments in the European Union (EU), where BSE countermeasures have been similar to those of Japan.

3. Details of the Request

Detail of the requests from MHLW is as follows:

1. The MHLW has proposed to change the present countermeasures against BSE by revising relevant regulations and/or requirements as follows.

   1) Countermeasures applied to domestic cattle (hereinafter referred to as "the domestic measures")
      a) To change an age limit required for domestic cattle to undergo BSE testing at slaughterhouses based on the Article 1, paragraph 7 of the Law on Special Measures against Bovine Spongiform Encephalopathy (Act No. 70 of 2002);
      b) To revise standards for hygienic removal of specified materials as required by the Act on Special Measures concerning Measures against Bovine Spongiform Encephalopathy, Article 2, paragraph 7 and Abattoirs act Article 2, paragraph 7, 1953; and
      c) To amend relevant requirements as required by the Food Sanitation Law (Articles 11 and 18) to ensure safety of foods including cattle vertebral column.

   2) Countermeasures on imports from the US, Canada, France and the Netherlands (hereinafter referred to as "the border measures")

a) To revise current import requirements applied to cattle meat and offal imported from the US and Canada; and
b) To revise current import requirements applied to cattle meat and offal imported from France and the Netherlands.

2. In the context of the proposal above, MHLW requested FSCJ specifically to address the following points:

1) For the domestic measures:
a) Age limit for BSE testing
To examine potential changes in BSE risks to human health due to the change of the age limit for BSE testing at slaughter from the current 20 months to 30 months of age.
b) Age limit for specified risk materials (SRMs)
To examine potential changes in BSE risks to human health due to the change of definition on age of SRMs (skull excluding tonsils, spinal cord and vertebral column) from "all ages" to "over 30 months of age".

2) For the border measures:
a) Age restriction on beef import
To examine potential changes in BSE risks to human health due to the change of the age restriction from the current 20 months to 30 months on cattle meat and offal imported from the US and Canada.
b) Age limits for SRMs in cattle
To examine potential changes in BSE risks to human health due to the change of definition on age of SRMs (skull excluding tonsils, spinal cord and vertebral column) from "all ages" to "over 30 months of age".
Note: As for imports from France and the Netherlands, the request was to examine potential changes in BSE risks to human health due to the change from the ban of import to allowing import under the age definition of SRM as "over 30 months of age".

3) Following completion of items 1) and 2) above, assessments are to be conducted on potential changes in BSE risks to human health due to further changes in the existing domestic and/or border measures, i.e. 1) a) and 2) a), in line with international standards for mitigating BSE risks.

4. The Process of the Risk Assessment

Upon the above mentioned requests from MHLW, FSCJ considered the items to be investigated in this assessment, and decided to conduct risk assessment through investigations on the following items. The outline of the process for it is shown in **Fig. 1**.

· The risk of live cattle, the risk of edible beef, and the risk of development of vCJD are investigated in this order in a similar way to the past risk assessments of BSE conducted by FSCJ.

· The risk is assessed at the stage of live cattle on the basis of BSE prion infectivity and BSE status of the cattle population.

· Infectivity of BSE prions is investigated based on tissue distribution of abnormal prion proteins (PrP^{Sc}) (accumulation sites: central nervous system (CNS) and other tissues) and the timing of deposition of PrP^{Sc} (effects of the dose of BSE prions in infection experiments, relationships between infection and onset, etc.) in experiments of oral infection.

· The status of infection in cattle is investigated based on the BSE status in the cattle population (age distribution and surveillance systems), risks of external challenge from other countries (the amount of imported live cattle, MBM, etc.), and domestic stability (the feed control measures, SRM removal and rendering conditions, preventive measures against cross-contamination, etc.). In the investigation, it is considered whether the approaches used in the self-tasking risk assessments is applicable to this risk assessment.

· The risk of the edible parts is estimated based on the situation of management in slaughterhouses (SRM removal, ban of pithing, BSE testing, age distribution of cattle slaughtered, etc.), and tissues and ages designated as SRM (cattle subjects of inspection and border measures).

· Atypical BSE in cattle, that is different from classical BSE, is investigated based on available data.

· Countermeasures against BSE in Japan is investigated for their effects on vCJD risks based on information on prevalence and epidemiology of vCJD.

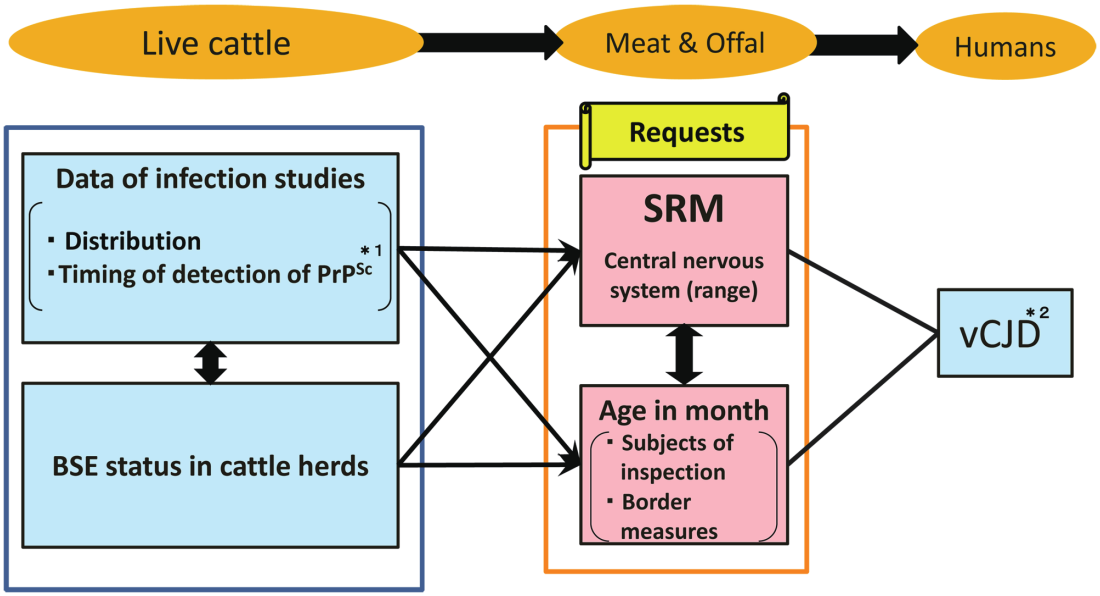


Fig. 1. Outline of the procedure of risk assessment
*1 PrP^{Sc}: Abnormal prion protein
*2 vCJD: Variant Creutzfeldt-Jakob disease

FSCJ considered certain conclusions to be drawn on 1) the domestic measures and 2) the border measures, based on up-to-date scientific information on BSE and the results of investigation on the BSE status, regulatory situations, etc.

Furthermore upon the MHLW's request for risk assessments on 1) the domestic measures and 2) the border measures at first and then on 3) further changes to the existing measures, FSCJ decided to conduct the risk assessments in this order.

II. BSE Prevalence and Its Control

1. Numbers of Cattle Tested and BSE Positives in Japan

Since 2001, the total number of cattle tested for BSE at slaughterhouses has amounted to 13.7 million, including 12.9 million cattle tested voluntarily by local governments and 0.8 million fallen cattle tested (2001–2011 (**Table 1**)). BSE tests conducted at slaughterhouses detected 21 BSE infected cattle. The total number of BSE positive cattle found in Japan is 36, including these 21 cases, 1 case found in Chiba prefecture in 2001, and 14 cases diagnosed by postmortem inspection at Livestock Hygiene Service Center[2]. Since February 2009, no BSE positive cattle have been identified (as of July 2012) **[**BSE screening test result from the website of MHLW: http://www.mhlw.go.jp/houdou/0110/h1018-6.html.**]**.

Data of birth year of the 36 cases indicate that the number of BSE cases by year of birth peaked in 1996 and 2000. No BSE cases have been found among cattle born after January 2002[2].

Data of cattle age at the time of BSE diagnosis showed that two cases of BSE were detected at their age of 21 and 23 months, that is younger than 30 months[2]. Infectivity in tissues from these two BSE cattle were studied using transgenic (Tg) mice over-expressing bovine prion protein (PrP), but no infectivity was observed[3,4].

2. Number of BSE Cattle Worldwide

The total number of BSE cases reported to Office International des Epizooties (OIE) is 190,629 (as of July 2012). The number of BSE cases peaked at 37,316 in 1992 and fell down remarkably to 45 cases in 2010 and 29 cases in 2011 **[Fig. 2**, The 69th Prion Expert Committee (March 23, 2012) material 2. The website of FSCJ: http://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20120323pr1&fileId=120**]**. This reflects not only the decrease in the number of

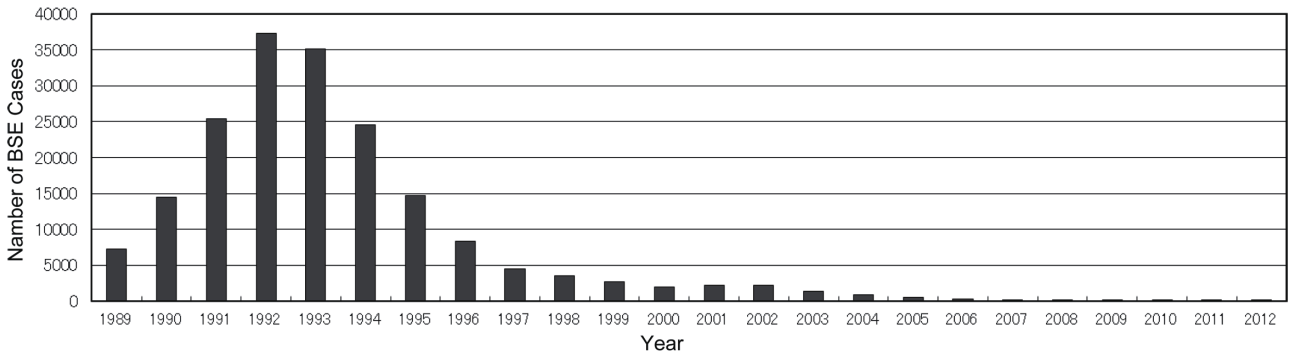
Table 1. Annual number of cattle tested for BSE in Japan

Year	Tested per year	Healthy slaughter	Fallen stock
2001	524,686	523,591	1,095
2002	1,258,126	1,253,811	4,315
2003	1,301,046	1,252,630	48,416
2004	1,364,276	1,265,620	98,656
2005	1,327,496	1,232,252	95,244
2006	1,313,034	1,218,285	94,749
2007	1,319,058	1,228,256	90,802
2008	1,336,304	1,241,752	94,452
2009	1,328,920	1,232,496	96,424
2010	1,321,899	1,216,519	105,380
2011	1,291,856	1,187,040	104,816
Total	13,686,701	12,852,252	834,349

Based on figures from the "BSE screening test result (the website of MHLW)" [*1] and the "BSE surveillance result (the website of MAFF)"[*2].

[*1] BSE screening test result from the website of MHLW: http://www.mhlw.go.jp/houdou/0110/h1018-6.html.

[*2] BSE surveillance result from the website of MAFF: http://www.maff.go.jp/j/syouan/douei/bse/b_sarvei/pdf/1206_survey.pdf.



	1992	…	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012(※1)	SUM
Total	37,316	…	2,215	2,179	1,389	878	561	329	179	125	70	45	29	7	190,629
Europe (Excluding UK, France, Netherlands)	36	…	716	769	616	469	293	189	95	74	46	26	17	5	4842
UK	37,280	…	1,202	1,144	611	343	225	114	67	37	12	11	7	1	184,619
France	0	…	274	239	137	54	31	8	9	8	10	5	3		1020
Netherland	0	…	20	24	19	6	3	2	2	1	0	2	1		88
U S A	0	…	0	0	0	0	1	1	0	0	0	0	0	1	3
Canada	0	…	0	0	2(※2)	1	1	5	3	4	1	1	1	—	20(※3)
Japan	0	…	3	2	4	5	7	10	3	1	1	0	0	0	36
Israel	0	…	0	1	0	0	0	0	0	0	0	0	0	—	1

Fig. 2. The number of BSE cattle worldwide

This table is based on the information of the website of OIE as of September 3, 2012.

[*1] The data of the UK (as of 6 July 2012), the US (as of 26 April 2012), and the other four countries were those reported in 2012.

[*2] One of which was detected in the US.

[*3] The total number of cattle tested in Canada includes one imported cattle and the first reported case in the US (December 2003).

BSE cases as a result of enhancement of feed controls in the UK where most of the world BSE cases have been reported, but also the reduction of BSE cases in other countries where feed control measures have also been enhanced.

The BSE status summarized above suggests that the BSE risk has been reduced to large extents in Japan and other countries through introduction and enhancement of feed control measures. The average age of cattle when diagnosed as BSE in the EU, where most of the world BSE cases have been found, increased from 76.3 months in 2001 to 151.7 months in 2010 in healthy slaughtered cattle. Also the age increased from 88.6 months in 2001 to 162.3 months in 2010 in high-risk cattle[5].

The number of cattle tested for BSE (2001–2010) accounted 96 million in the EU and in other regions (**Table 2**).

3. BSE Testing System in Each Country

Table 3 shows BSE testing system in each country.

BSE testing of cattle destined for human consumption is conducted for cattle aged 21 months or older in Japan[6], whereas cattle aged 72 months or older is tested in the EU with exceptions in some countries (Note: In 2014, BSE testing covers risk cattle over 24 months and healthy slaughtered cattle over 72 months in most of EU countries.)[7,8].

Although BSE surveillance is in place in the five countries, conditions, symptoms, and ages of cattle to be tested for high-risk cattle are different among countries.

4. Definition of SRMs in Each Country

Table 4 shows the definition of SRMs in each country.

In Japan, SRMs are defined for cattle of all ages, while in the US, Canada, the EU and as stipulated in the OIE regulation, some age limits are set for the CNS as SRM. Mesentery and intestines from the duodenum to the rectum derived from cattle of all ages are defined as SRMs in the EU, while the distal ileum, but not the other intestines nor mesentery, from cattle of all ages are defined as SRMs in other countries. The tonsils of cattle aged 30 months or older are defined as SRMs in Canada, but in other countries, those of all ages are defined as SRMs.

Table 2. The number of cattle tested for BSE in the EU and in other countries

Year	Total number	Healthy slaughter	Fallen stock	Emergency slaughter	Clinical signs	BSE suspects	BSE culling
2001	8,516,227	7,677,576	651,501	96,774	27,991	3,267	59,118
2002	10,423,882	9,124,887	984,973	182,143	71,501	2,658	57,720
2003	11,008,861	9,515,008	1,118,317	255,996	91,018	2,775	25,747
2004	11,081,262	9,569,696	1,151,530	233,002	107,328	3,210	16,496
2005	10,145,325	8,625,874	1,149,356	266,748	86,826	2,972	13,549
2006	10,152,335	8,663,348	1,309,132	105,898	66,695	2,344	4,918
2007	9,737,571	8,277,202	1,313,959	103,219	39,859	1,861	1,471
2008	10,071,873	8,499,780	1,450,365	76,616	41,655	2,352	1,105
2009	7,485,918	6,294,547	1,110,975	59,594	18,906	844	1,052
2010	7,515,151	6,330,807	1,104,532	58,323	20,451	660	378
Total	96,138,405	82,578,725	11,344,640	1,438,313	572,230	22,943	181,554

Note) 2001, 2002: only EU15, 2003: EU25 and Norway

2004, 2005: EU25, Bulgaria, and Norway

After 2006: EU27 and Norway

Based on the report on the monitoring and testing of ruminants for the presence of Transmissible Spongiform Encephalopathy (TSE) in the EU[5].

Table 3. Testing system in each country (as of July 2012) [*4]

	Japan	The US/Canada	France/ The Netherlands	OIE (reference)
Meat inspection (healthy slaughtered cattle, etc)	Cattle aged 21 months or older (local governments voluntarily conduct BSE testing on cattle aged 20 months or younger)	-[*3]	Cattle aged 72 months or older	-
Monitoring of BSE prevalence [*1] (high-risk cattle[*2])	Fallen cattle aged 24 months or older (cattle aged 24 months or younger that exhibit symptoms showing CNS impairment are covered)	High-risk cattle aged 30 months or older and cattle of all ages with symptoms showing CNS impairment suspected of BSE	High-risk cattle aged 24 months or older (France) and aged 48 months or older (The Netherlands)	High-risk cattle aged 30 months or older

[*1] Testing for continuous surveillance and investigation of the BSE status.

[*2] Testing for fallen cattle, cattle with symptoms showing CNS impairment, and downers.

[*3] OIE standards do not require BSE screening tests.

[*4] The 67th Prion Expert Committee (January 19, 2012) material 2 was partially revised.

[The website of FSCJ: www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20120119pr1&fileId=210.]

Table 4. Definition of specified risk materials (SRMs) in each country*

Country	SRMs
Japan	• The head (excluding the tongue and cheek meat), spinal cord, and distal ileum (two meters from the connection to the caecum) of cattle of all ages. • The vertebral column (excluding transverse processes of thoracic and lumbar vertebrae, vertebrae of the tail and the wings of sacrums) of cattle of all ages.
The US	• The brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding tail vertebrate, thoracic and lumber transverse processes, and sacral wings), and dorsal root ganglia of cattle aged 30 months or older. • The tonsils and distal ileum of cattle of all ages.
Canada	• The skull, brain, trigeminal ganglion, eyes, tonsils, spinal cord, and dorsal root ganglia of cattle aged 30 months or older. • The distal ileum of cattle of all ages.
EU (France and the Netherlands)	• Skull (excluding the mandible but including the brain and eyes) and spinal cord of cattle aged over 12 months. • Vertebral column (excluding the vertebrae of tail, spinous and transverse processes of cervical, thoracic and lumbar vertebrae, the median sacral crest, and the wings of sacrum but including the dorsal root ganglia) of cattle over 30 months of age. • The tonsils, the intestines from duodenum to the rectum and mesentery of cattle of all ages.
OIE (countries with controlled risk)	• The skull (including the brain and eyes), spinal cord, and vertebral column of cattle aged 30 months or older. • Tonsils and distal ileum of cattle of all age.

* The 67th Prion Expert Committee (January 19, 2012) material 2 was partially revised.

[The website of FSCJ: http://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20120119 pr1&fileId=210.]

5. Feed Control Measures in Each Country

Table 5 shows cattle materials prohibited from use in livestock feed.

Feed control measures were enhanced in France in November 2000[9] as well as in the Netherlands in December 2000[10] to prevent cross-contamination and to prohibit the use of MBMs produced from livestock (cattle, pigs, and poultry) as feed for these livestock. Similarly, in Japan in October 2011, the use of MBMs produced from livestock (cattle, pigs, and poultry) was prohibited for use as feed for these livestock. However, the measures for the use of the following

Table 5. Materials in cattle that are prohibited for use in livestock feed (as of July 2012)

Tissues		Japan		The US		Canada		France and the Netherland	
		Ruminants	Animals other than ruminants	Ruminants	Animals other than ruminants	Ruminants	Animals other than ruminants	Ruminants	Animals other than ruminants
Brain	At or over 30 months of age	×	×	×	×	×	×	×	×
	Under 30 months of age	×	×	×	○	×	○	×	×
Spinal cord	At or over 30 months of age	×	×	×	×	×	×	×	×
	Under 30 months of age	×	×	×	○	×	○	×	×
Skull		×	×	×	○	×	×*	×	×
Eyes		×	×	×	○	×	×*	×	×
Trigeminal ganglia		×	×	×	○	×	×*	×	×
Vertebral column		×	×	×	○	×	×*	×	×
Dorsal root ganglia		×	×	×	○	×	×*	×	×
Tonsils		×	×	×	○	×	×*	×	×
Distal ileum		×	×	×	○	×	×	×	×

Cross: Not acceptable for feed

Circle: Acceptable for feed (Tissues from BSE positive cattle are not acceptable for use in feed)

* In Canada, materials in cattle that are prohibited for livestock feed for non-ruminants are materials from cattle aged 30 months or older, excluding the distal ileum **[**The 72nd Prion Expert Committee (Tuesday, June 26, 2012) material 4-1. The website of FSCJ: http://www.fsc.go.jp/fsciis/attachedFile/download?retrievalId=kai20120323pr1&fileId=12.**]**.

MBMs were lifted upon meeting some requirements in November 2001 and 2005: the use of MBMs produced from poultry (chicken meal and others) for feed for poultry and pig in 2001; and the use of MBMs produced from pig for feed for poultry and pig in 2005[11]. The use of brains and spinal cords of cattle aged 30 months or older for livestock feed was prohibited in Canada in July 2007[12] and in the US in October 2009[13,14]. In the US, control measures for SRMs for feed are stricter than those for meat as food.

III. Findings of Infection Experiments and Other Studies

1. Findings of Experimental Oral Infection with BSE Prions

Tissue distribution of PrP^{Sc}, infectivity of each tissue **[**the infectivity of prions in various tissues was determined based on the period from the time of intracerebral (i.c.) and intraperitoneal (i.p.) inoculation of cattle tissues to mice until the time of detection of PrP^{Sc} in the mice.**]** and dose-related incidence rate and incubation period in cattle have been reported based on the findings of experimental oral infection of calves with BSE prions. The results of experiments of oral infection performed in the UK until 2005 have been described in "Measures against Bovine Spongiform Encephalopathy (BSE) in Japan −Interim report−"[15], "Food Safety Risk Assessment Related to Measures Against Bovine Spongiform Encephalopathy (BSE) in Japan"[16], and "Food Safety Assessment on Equivalency of the BSE Risk by Ingestion of Cattle Meat and Offal Controlled under Export Programs for Japan in the US/Canada and the BSE Risk by

Ingestion of Meat and Offal derived from Japanese Cattle"[17]. The new findings, mainly obtained after these studies, are summarized as follows:

1) Tissue distribution of PrP^{Sc} and infectivity in cattle

a) Studies performed by the UK group

The Veterinary Laboratories Agency (VLA) conducted bioassays using wild-type mice (RIII mice and C57BL/6 mice) to study the infectivity in each tissue of Friesian-Holstein cattle orally exposed to the brainstem of BSE cases identified in the UK. In this study, 30 calves of 4 months old were each dosed orally with a pooled homogenate of 100 g of the BSE-affected brainstem **[**infectivity (infectious titer) measured in RIII mice = $10^{3.5}$ RIII mouse i.c./i.p. 50% infective dose (ID_{50}) per 1 g of cattle tissue)**]**. The challenged cattle were sequentially slaughtered from 2 to 40 months post-exposure, and tissues from the slaughtered cattle were removed for detection of PrP^{Sc} by immunohistochemistry (IHC) and mouse bioassay. PrP^{Sc} was detected by IHC in the medulla oblongata of the challenged cattle at 32 months post-exposure. In detail, PrP^{Sc} was detected in one of the two, three of the three, two of the three, and one of the two cattle at 32, 36, 38, and 40 months post-exposure, respectively. PrP^{Sc} was not detected in any of the three cattle killed at 18 months, in any of the three killed at 22 months, and in one killed at 26 months post-exposure. The infectivity was not detected in tissues of the caudal medulla oblongata, spinal cord, and dorsal root ganglion (DRG) until 26 months post-exposure, but was detected at 32 months post-exposure, by mouse bioassay in which 0.02 mL and 0.1 mL of a 10% homogenate of each tissue was inoculated into the mouse brain and abdominal cavity, respectively[18,19].

Arnold *et al*. estimated the infectious titer by mouse (RIII) bioassay for various tissues taken from cattle dosed orally with the pooled homogenate of 100 g of the BSE-affected brainstem used in the above oral exposure study. In their study, one to three cattle were each slaughtered at 2, 6, 10, 14, 22, 26, 28, 32, 36, 38, and 40 months after oral exposure, and 10% homogenates of their CNS, DRG, and distal ileum were each pooled as inocula at each sequential slaughtering time point. The detection limit of infectious titer in RIII mice was estimated to be $10^{-1.3}$ RIII mouse i.c./i.p. ID_{50}/g. The infectivity was estimated to be lower in the DRG than in the CNS by approximately 10 RIII mouse i.c./i.p. ID_{50}/g, and to be lower in the thoracic and cervical DRG than in the CNS by approximately $10^{1.5}$ RIII mouse i.c./i.p. ID_{50}/g. The infectivity in the distal ileum was detectable from 6 months post-exposure, increased at 14 to 18 months, and then decreased to non-detectable levels at 36 months, followed by an increase from 38 to 40 months. Thus the infectivity of the distal ileum changed with the period post-exposure. The estimated 95% confidence interval of infectious titer in RIII mice was $10^{-1.12}$ to $10^{1.94}$ RIII mouse i.c./i.p. ID_{50}/g. The maximum infectious titer was $10^{1.59}$ RIII mouse i.c./i.p. ID_{50}/g on average at 14 months post-exposure, and estimated to be equivalent to $10^{-1.21}$ cattle oral ID_{50}/g ($10^{-1.21}$ ID_{50} in cattle with oral administration).

In their study, also the infectivity in medulla oblongata was determined by RIII mouse bioassay using a pooled 10% tissue homogenate from cattle at each kill time point. Infection to the mice was not observed in the tissue from cattle at 22 and 26 months post-exposure, but was detected at 32, 36, 38, and 40 months post-exposure with the infectivity increasing with age. From these observations, the authors estimated the infectious titer of CNS at 32 months post-oral exposure of the pooled homogenates of 100 g of the BSE-affected brainstem to be $10^{-2.7}$ cattle oral ID_{50}[20].

Wells *et al*. performed cattle bioassay to determine the infectivity of tissues from the cattle orally exposed to the brainstem of the BSE cases identified in the UK. The authors killed the orally exposed cattle at sequential time points from 6 to 36 months post-exposure. A pooled tissue homogenate was prepared for each of the CNS, intestines, liver, kidney, thymus, mesenteric lymph nodes, tonsils, muscles, and other tissues at each time point. One ml of 10% pooled brainstem homogenate was inoculated into the brain of Holstein-Friesian calves (five calves per group) aged 4 to 6 weeks. After being inoculated, the calves were monitored for clinical signs of disease. In addition, the accumulation of PrP^{Sc} in the brain was examined by immunological methods such as enzyme-linked immuno-sorbent assay (ELISA), IHC, and Western blotting (WB). The medulla oblongata and spinal cord tissues taken from the orally exposed cattle at 6, 10, 18, and 26 months post-exposure did not cause any sign of BSE infection in the intracerebrally inoculated calves during the observation period of 90 months. Consistently, PrP^{Sc} was not detected in the brains of these calves by any immunological methods. However, the medulla oblongata and spinal cord taken from the orally exposed cattle at 32 months post-exposure induced BSE signs in all the five intracerebrally inoculated calves at 22 to 24 months post-inoculation (mpi). In addition, PrP^{Sc} was detected in the brain of these calves by all the immunological methods. The distal ileum derived from the orally exposed cattle at 6, 10, and 18 months post-exposure infected all the intracerebrally inoculated calves with PrP^{Sc} detected in their brain. In one of the five calves inoculated with palatine tonsils from the orally exposed cattle at 10 months post-exposure, symptoms of BSE developed and PrP^{Sc} was detected in their brain. On the other hand,

any other tissues did not induce any BSE symptoms in intracerebrally inoculated calves during the observation period from 65 to 98 mpi, nor accumulation of PrP^{Sc} in their brain[18,21,22].

It has been estimated that one cattle oral ID_{50} is equivalent to $10^{2.8}$ RIII mouse i.c./i.p. ID_{50} while one RIII mouse i.c./i.p. ID_{50} is equivalent to $10^{2.7}$ cattle i.c. ID_{50}. One oral cattle ID_{50}, therefore, is estimated to be equivalent to $10^{5.5}$ cattle i.c. ID_{50}[23].

In another study, VLA investigated the relationship between oral dose of the BSE-affected brainstem homogenate and the period from administration to deposit of detectable PrP^{Sc} in cattle. In this study, 100 g or 1 g of the BSE-affected cattle brainstem homogenate (infectious titer = approximately $10^{3.1}$ RIII mice i.c./i.p. ID_{50}/g) was orally administered to calves aged 4 to 6 months (100 calves for each dose). The calves were slaughtered sequentially from 4 months until 60 or 89 months post-exposure for examination of PrP^{Sc} in the CNS and related peripheral nerve ganglion. In the group of 100 g administration, PrP^{Sc} was first detected in the brain of one of the six animals at 30 months post-exposure, although clinical signs were not yet observed. In the group of 1 g administration, PrP^{Sc} was detected first at 44 months in the brain of one animal with clinical signs. However, PrP^{Sc} was not detected in the brains of any of six animals killed at 27 months after 100 g administration, nor at 42 months after 1 g administration[24–26].

Stack *et al*. studied by IHC the distribution of PrP^{Sc} in the duodenum, central jejunum, and distal ileum that were derived from the above VLA studies. In the 100 g administration group, PrP^{Sc} was detected in the medulla oblongata and in a part of the lymph follicles of ileum and jejunum at 33 months after administration. PrP^{Sc} was detected in a part of ileum samples at 4 months post-exposure, earlier than the time point of detection in the CNS, and subsequently during the observation period. PrP^{Sc} was also detected in the jejunum at 4 to 30 months post-exposure, but not in the duodenum during the same period. The rate of detection of PrP^{Sc} (the number of positive cattle over the number of tested cattle) in the jejunum and ileum for the entire period were 8/58 (13.8%) and 45/99 (45.5%), respectively. The average frequencies of positive lymph follicles in the jejunum and ileum per an animal were 1.47% and 1.26%, respectively, in cattle where PrP^{Sc} was detected in their intestines. The number of lymph follicles decreased, but the ratio of the number of PrP^{Sc} positive lymph follicles to the total number of lymph follicles increased with age in the PrP^{Sc} positive ileum. The number of cattle having PrP^{Sc} positive lymph follicles in their intestine decreased with age.

In the 1 g administration group, PrP^{Sc} was detected only in ileal lymph tissues from one of the 98 cattle at 24 months post-exposure. PrP^{Sc} was hardly detected in the enteric nerve tissues. The proportion of PrP^{Sc} positive lymph follicles was lower in the 1 g administration group than in the 100 g administration group. PrP^{Sc} was not detected in the jejunum and duodenum of the 1 g administration group. From these results, the authors considered that in experimentally-infected BSE cattle, the infectivity in the small intestine other than the ileum is lower than that in the ileum regardless of the exposure level[25].

b) Experimental studies in Germany

In studies conducted by the Friedlich-Loeffler-Institut in Germany, Tg mice over-expressing bovine PrP (TgbovXV) were used. The susceptibility of the mice was examined by i.c. inoculation of BSE-affected brainstem of cattle, and demonstrated to be 10,000 times higher than that of RIII mice and approximately 10 times higher than that of cattle[27].

To delineate sequential profiles of PrP^{Sc} deposits in cattle body, Hoffmann *et al*. administered orally 56 Simmental cross-bred calves aged 4 to 6 months with a pooled homogenate of brainstem from BSE cases (infectious titer measured by TgbovXV mice was $10^{6.1}$ i.c./i.p. ID_{50}/g) at a dose of 100 g brainstem. Four to five calves were killed every 4 months, and more than 150 samples of tissues and fluids were taken from them. PrP^{Sc} was not detected in the brain until 20 months after oral administration[1].

They studied also the distribution of PrP^{Sc} and infectivity in intestinal tissues from these cattle during incubation period. The distribution of PrP^{Sc} in the jejunum, ileum, and ileocecal junction, which were collected to include Peyer's patches (PPs), was studied by rapid tests with IHC and ELISA, and PTA-WB [PTA-WB: WB test where PrP^{Sc} is selectively enriched using sodium phosphotungstic acid, the sensitivity of which is increased compared with that of WB without enrichment.]. Infectivity in each tissue of the calves killed at 8 to 20 months post-exposure was examined by bioassay using TgbovXV mice, which were intracerebrally inoculated with a 10% homogenate of the tissues at a volume of 30 μL per mouse. In the orally exposed cattle, clinical signs were observed first at 32 months post-exposure. The intestinal tissues of all three calves killed at 1 month post-exposure were PrP^{Sc} negative. The accumulation of PrP^{Sc} and infectivity were detected in the ileum taken from 40 out of 43 calves killed at 4 to 44 months post-exposure. The distribution of PrP^{Sc} and infectivity were observed in jejunum, ileum, and ileocecum in relatively young calves at 8 months post-exposure, in more wide area at 12 months post-exposure, compared in calves killed later. IHC demonstrated the distribution of PrP^{Sc} positive-lymph follicles mainly in the ileum but not in the jejunum.

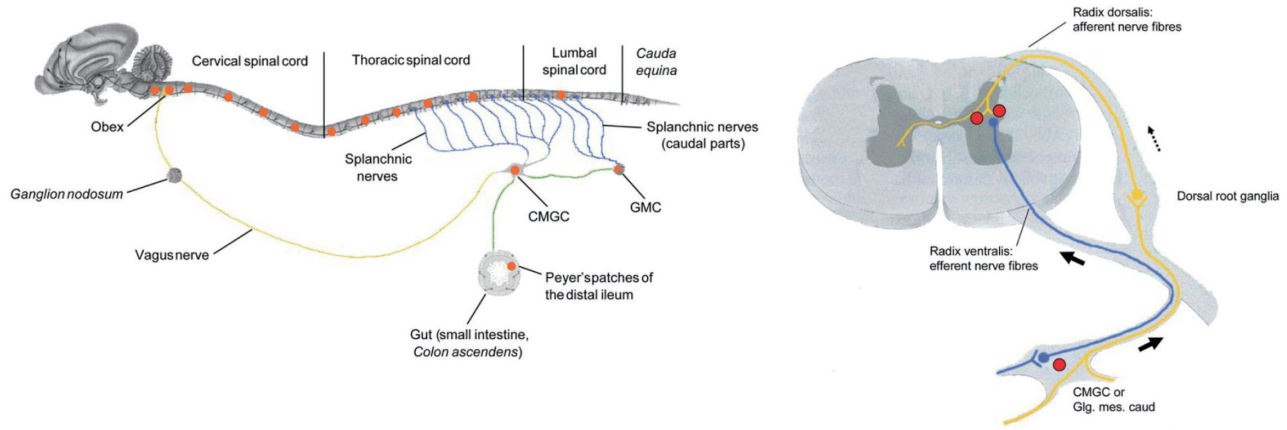


Fig. 3. Probable migration route of BSE prions from the intestine to the brain. Based on the literatures of Hoffmann C. et al.[1)]

Mouse bioassay demonstrated the presence of the infectivity in the ileum PP in 11 of the 16 calves at 8 to 20 months post-exposure with the infection rate of the inoculated mice being 23 to 87%. The presence of infectivity was also confirmed in the jejunum PP in 7 of the 16 calves at 12 months post-exposure, but the infection rate of the mice inoculated with these tissues was 13% on average. IHC showed time sequential changes in the number of PrP^{Sc} positive cells, and the presence of PrP^{Sc} in tingible body macrophages (TBM) [Macrophages found in germinal centers of the thymus, spleen, and lymph nodes as stainable macrophages containing chromatin fragments.] of the ileum[28)].

In addition, in one calf killed at 24 months and that killed at 28 months post-exposure, PrP^{Sc} was detected in their obex, although they did not show any clinical signs. Their gut-associated lymphoid tissues, tonsils, retropharyngeal lymph node, spleen, most part of the sympathetic and parasympathetic nerve systems, nerve fibers, nerve ganglion, and brain were examined for PrP^{sc} accumulation by IHC. As a result, PrP^{Sc} was detected in the medulla oblongata at the level of obex, and in the bridge, spinal cord, coeliac ganglion, caudal mesenteric ganglion, and ileum from the calf killed at 24 months post-exposure. On the other hand, PrP^{Sc} was detected only in the medulla oblongata at the obex, but not in the other tissues, in the calf killed at 28 months. These results indicated that PrP^{Sc}, when administered in a large amount, could reach the brain at 24 months after administration. The authors considered that the orally administrated prions may invade from the intestinal tract, and then reach the CNS via nerves, but not via lymphoreticular systems. They suggested a route running from the intestinal membrane ganglion complex through the nerves of viscera and lumbar/caudal thoracic spinal cord (sympathetic innervation of digestive canal) or a route running via vagal nerve (parasympathetic innervation of digestive canal) (**Fig. 3**). Moreover, the authors inferred that prions migrate to the DRG and peripheral nerves after reaching the CNS[1)].

To study the mechanism of BSE onset, Kaatz *et al*. orally administered 56 Simmental cross-bred calves with a pooled homogenate of brainstem from BSE cases (infectious titer measured by TgbovXV Tg mice was $10^{6.1}$ ID_{50}/g) at a dose of 100 g brainstem, intermittently slaughtered two to five calves every 4 months from 16 to 44 months after administration, and collected more than 150 samples of tissues and fluids. The authors examined the accumulation of PrP^{Sc} in the cattle by IHC, and conducted bioassay using TgbovXV Tg mice (15 mice per group) with i.c. inoculation of 30 µL of 10% tissue homogenate. Possible or probable clinical signs were observed in any of two calves at 32 months post-exposure, and the accumulation of PrP^{Sc} at the obex was detected in one of the two calves slaughtered at 28 months but not in calves slaughtered at 24 months post-exposure. On the other hand, infectivity of the caudal medulla was observed at 24 months post-exposure in one of the 7 inoculated mice. PrP^{Sc} was detected in the lymph follicles and intestinal nerve system in the distal ileum through the entire test period. Infectivity in the sympathetic and parasympathetic nerve ganglion at 16 and 20 months post-exposure was demonstrated by the mouse bioassay, but PrP^{Sc} was not detected in them by IHC. Also, the transient presence of infectivity in the thoracic spinal cord (T7) at 16 months post-exposure was shown by the mouse bioassay, but PrP^{Sc} was not detected in this tissue by IHC. According to the authors' view, these results confirm the two routes, the sympathetic and parasympathetic routes, for orally ingested BSE prions to reach the CNS[29)].

c) Studies in Japan

In Japan, the National Institute of Animal Health has conducted laboratory studies on BSE prions. Okada *et al*. orally administered 28 Holstein-Friesian or crossbred calves (aged 3 to 11 months) with 5 g of brainstem of BSE cattle

as a pooled 10% homogenate of the tissues of 10 cattle from VLA. Infectious titer of the inocula was approximately $10^{6.7}$ i.c. LD_{50}/g **[**LD_{50}: Lethal Dose 50 (The amount statistically estimated to kill half of test animal population within a few days.)**]** in TgBoPrP mice[30] **[**Tg mice overexpressing bovine PrP. They are 10-fold or 1000-fold susceptible to PrP^{Sc} compared to cattle or RIII mice, respectively[4]**.]**. The challenged calves were slaughtered sequentially at selected time points post-exposure, and the tissues of cervical, thoracic, and lumbar spinal cords, sacral cord, small intestine, etc, were removed and examined for the distribution of PrP^{Sc} by IHC and WB. PrP^{Sc} was not detected in the CNS of cattle killed from 18 to 30 months post-exposure, but was detected in the CNS of 7 out of the 15 cattle killed at or after 34 months post-exposure; 5 out of the 7 PrP^{Sc} positive cattle showed early stages of clinical signs (3 calves were slaughtered each at 34, 42, and 58 months, and 2 calves at 66 months post-exposure). In two cattle killed at 36 and 48 months, no clinical signs were observed, but small accumulation of PrP^{Sc} was detected in the dorsal motor nucleus of the vagus nerve, trigeminal nucleus at the level of the obex, intermediolateral nucleus of T13, etc.

Distribution of PrP^{Sc} in the small intestine in three meters length from the ileocecal junction was examined by immunological detection of PrP^{Sc} in tissues taken at 50 cm intervals that contain a continuous Peyer's patch (CPP) and discrete Peyer's patches (DPPs). PrP^{Sc} was detected in the CPP, but not in the DPPs, of the posterior small intestine (three meters from the ileocecal) in five calves (three at 20 months, one at 30 months and one at 46 months post-exposure) without clinical signs. The rates of detection of PrP^{Sc} in the follicles of the above five cattle were each 2.18% (9/413), 0.07% (1/1447), 0.26% (2/762), 0.23 (3/1282), and 1.0% (2/200). PrP^{Sc} positive cells in the follicles were identified as TBM. Infectivity of the CPP was detected by bioassay using TgBoPrP mice (five mice per group), which were intracerebrally inoculated with 20 μL of 10% homogenate of the CPP containing PrP^{Sc} positive follicles collected from the cattle at 20 months post-exposure. The average incubation period in the CPP-inoculated mice was 248.9 ± 14.4 days. On the other hand, mice inoculated with DPPs showed no infectivity and survived for 650 days[31].

Fukuda *et al*. investigated a relationship between PrP^{Sc} accumulation in the CNS and development of clinical signs in cattle infected with the BSE agent. In their study, 16 calves (Holstein, four to eight calves per group) were intracerebrally inoculated with 1 mL of 10% homogenate of brain from two BSE cases found in Japan and one case in the UK, then monitored clinically and examined for PrP^{Sc} accumulation in the CNS by IHC and WB. In the group inoculated with the brain of the UK case, PrP^{Sc} was not detected in the CNS at three months post-inoculation, but was detected at ten months by both IHC and WB. Vacuolar degeneration was observed at 16 months, and clinical signs developed from 18 months. In the group inoculated with the brain of a Japanese case (BSE/JP6), PrP^{Sc} was detected in the CNS at 12 months and clinical signs were observed at 19 months[32].

d) Other studies

Espinosa *et al*. studied infectivity in tissues of asymptomatic cattle using Tg mice over-expressing bovine PrP (BoPrP-Tg110 mouse). In their study, 15 calves (four to six months of age) were orally challenged with 100 g of the brainstem homogenate derived from 150 cattle showing clinical signs (pooled homogenate shared by VLA). Three challenged calves were each slaughtered at 20, 24, 27, 30, and 33 months post-exposure, and tissues and fluids were taken from them and pooled together at each kill time point. After PrP^{Sc} accumulation was examined by ELISA and WB, 20 μL of 10% homogenate of each tissue or fluid taken from asymptomatic cattle at the indicated times were intracerebrally inoculated to BoPrP-Tg110 mice to examine the infectivity of the homogenates. The inoculated mice were examined for incubation period and survival time.

Symptoms were not observed in any of the cattle orally exposed to the BSE-affected brainstem homogenate until 33 months post-exposure. Accumulation of PrP^{Sc} was detected in the brainstem taken from cattle at 33 months post-exposure by ELISA test, but the accumulation in the brainstem from other cattle was not detected in any test. The infectivity was detected by bioassay using the transgenic mice in the brainstem, sciatic nerve, ileum (PP), and tonsils, but not in the spleen, muscles (the location is unknown), blood, and urine from the challenged cattle. The earliest time point when infectivity was detected in the cattle brainstem by the mouse bioassay was 27 months post-exposure. The rates of infection in mice (the number of PrP^{Sc}-positive mice / the number of inoculated mice) inoculated with the cattle brainstem at 27, 30, and 33 months post-exposure, were 2/6, 2/6, and 6/6, respectively, increasing sharply at 33 months. The rate of infection in mice inoculated with the sciatic nerve taken from cattle either at 30 or at 33 months post-exposure was 1/5. The PPs and tonsils collected from cattle at any time points showed infectivity in mice with the rate of infection of 1/5 to 3/5 and 1/6 to 1/5, respectively. From these findings, the authors concluded that the BSE agent propagates through the nervous system in asymptomatic cattle[33].

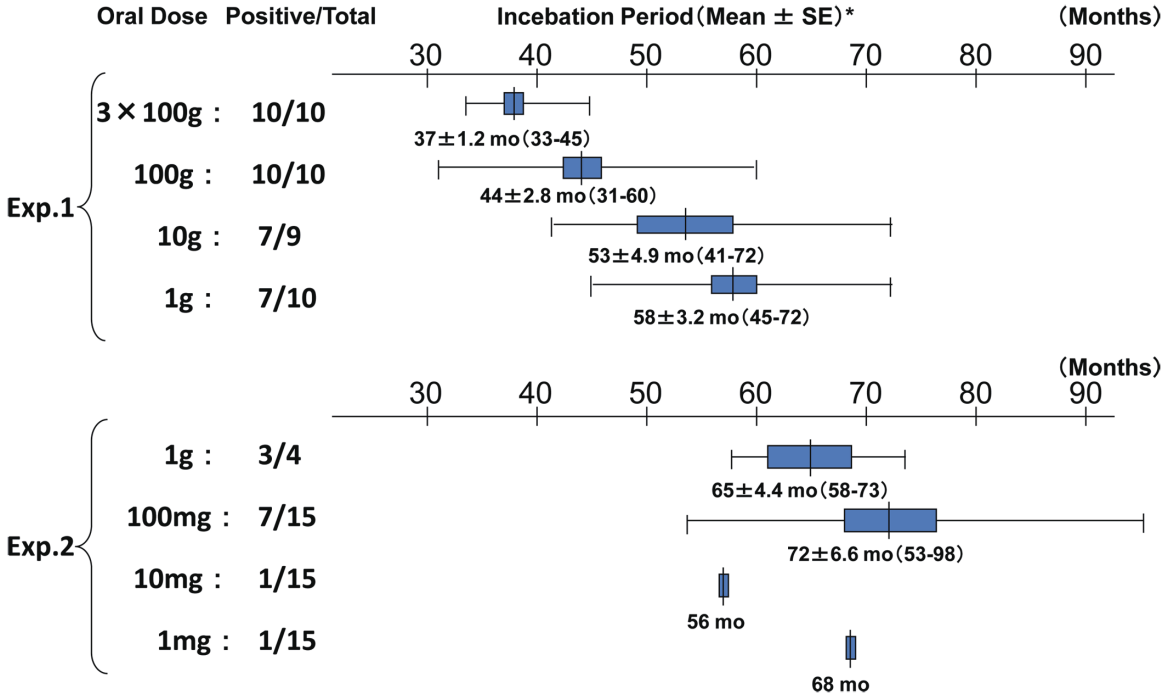


Fig. 4. Summary of data on attack rates and incubation periods at various doses
* The range of months from exposed to BSE onset clearly confirmed from clinical signs.
Based on literatures[23] of Wells *et al*.

2) Oral doses and incubation period in cattle

VLA studied the incidence and incubation period of BSE in relation to inoculation dose of the BSE agent in cattle[23]. In one of their experiments (designated as Experiment 1), ten calves (Holstein-Friesian, Friesian cross bred, and Aberdeen Angus-Jersey crossbred) received a single oral inoculation of a pooled homogenate of BSE-affected cattle brainstem (infectious titer measured using RIII mice is $10^{3.5}$ i.c./i.p. ID_{50}/g) at a dose of 100 g, 10 g or 1 g brainstem, or three-days repeated oral inoculations of the same homogenate at a dose of 100 g per once. In the other experiment (designated as Experiment 2), 15 calves received a single oral inoculation of 0.1 g, 0.01 g, or 0.001 g of the same brainstem as a pooled homogenate, and five calves received 1 g. The rate of BSE incidence and incubation period **[**The period from administration to onset**]** of these challenged calves were analysed in relation to inoculation dose.

The animals were killed when judged to be clinically affected. Otherwise, they were kept under the observation until 110 months post-exposure. Clinical signs were detected first at 31 months post-exposure in the animals challenged with 100 g of the brainstem, while the signs were detected first at 45 months post-exposure in the animals challenged with 1 g of the brainstem. Data on the dose, the incidence rate and the incubation period were summarized in **Fig. 4** to show their relationships.

Based on approximation with a log-normal distribution of the relationship between doses and attack rates, one oral cattle ID_{50} was calculated to be almost equivalent to $10^{2.8}$ i.c./i.p. mouse ID_{50}, and a dose of 0.20 g of the inoculated brainstems (95% confidence interval: 0.04 to 1.00 g) was estimated to affect clinically 50% of cattle. In addition, as shown in **Fig. 4**, there were wide variations in incubation period among the animals received the same dose. Nevertheless, the incidence rate (the number of BSE positive animals / the number of exposed animals) decreased with the decrease in dose. The approximation with a log-normal distribution indicated that the average incubation period was reduced with the increase in dose. However, the minimum infection dose could not be established, because onset of BSE was noted in an animal inoculated with the lowest dose (0.001 g of the brainstem of BSE cattle)[23].

The incubation period of BSE has been estimated by different authors on the basis of the epidemiological studies conducted in the UK as follows. Wilesmith *et al*. estimated the incubation period to be 2.5 to 8 years based on simulation of BSE in the UK until 1987 under the assumption that incubation periods and ages of BSE cattle were log-normally distributed[34]. Ferguson *et al*. predicted the average incubation period to be 4.75 to 5.00 years (95% confidence interval) by back-calculation method based on the UK birth cohort data of each year from 1981 to 1992 including estimated postnatal age-in-month[35]. Arnold *et al*. estimated age-dependent BSE risk by back-calculation methods based on birth cohort data

of dairy cattle in the UK from 1984 to 1995, where the highest risk was predicted to be at 6 months of age and the average incubation period was estimated to be 5.5 years[36]. From the relationship between orally inoculated doses and average incubation periods observed in the above inoculation experiments, Wells *et al*. estimated the amount of PrP^{Sc} given to field cases in the UK to be 100 mg to 1 g in terms of a single dose of the brainstem derived from the UK BSE cases. This estimates were based on the incubation period of 5 to 5.5 years, which was estimated for field BSE cases in the UK by epidemiological analyses. They pointed out, however, that precise estimation of the amount consumed by cattle in field was difficult due to a large variation of incubation period even among calves exposed experimentally to a single dose[23].

To estimate the dose-related timing when PrP^{Sc} is detected in the CNS and related peripheral nerve ganglions, Arnold *et al*. conducted a logistic regression analysis based on the data of experimental studies with cattle conducted in VLA[18,23,24,26]. The timing of detection of PrP^{Sc} in 50% of the cattle (for more details, refer to III. 1. 1) a) Studies performed by the UK group) was estimated to be 1.7 months (95% confidence interval: 0.2 to 4.0 months) and 9.6 months (95% confidence interval: 4.6 to 15.7 months) before clinical onset in the 1 g and 100 g administration groups, respectively, indicating that the interval between PrP^{Sc} detection and clinical onset is shorter in the 1 g than in the 100 g group. Although confidence intervals were wide, there was a correlation between the frequency of detection of PrP^{Sc} in the medulla at the level of obex and the period elapsed after exposure. There was a significant difference in the ratio of incubation period to the period of detection of PrP^{Sc} after exposure between the 100 g and 1 g dose groups, when compared on the basis of the result of the above analysis. It was estimated that PrP^{Sc} is detected in 50% of exposed animals at the time point elapsed 79% and 97% of incubation period after exposure in the 100 g and 1 g groups, respectively. For the 100 g dose group, PrP^{Sc} was initially detected in the medulla at the obex then in the cervical and thoracic spinal cords approximately one month post-exposure, and in the midbrain and lumber spinal cord approximately 1.3 months after the initial detection in the medulla. In view of epidemiological observations in the UK[34], the authors considered that the experimental observations with the 1 g dose group may correspond to situations of field BSE cases, and that PrP^{Sc} might be detected in the medulla at the level of obex in field-infected BSE cattle approximately 1.5 months prior to clinical onset[24].

Based on the experimental results on the relationship between the dose and incubation period, and on EU regulations for SRM and feed, the European Food Safety Authority (EFSA) concluded that the data of incubation periods of the 1 g dose group should better conform to field situations compared to that of the 100 g administration group[37].

Simmons *et al*. compared the timing of PrP^{Sc} detection by histological observation and IHC in tissues of the midbrain, rostral medulla oblongata, obex, cervical spinal cord, thoracic spinal cord, lumber spinal cord, cervical and thoracic DRG, and cranial cervical ganglion, stellate ganglion, and trigeminal ganglion obtained in the studies of VLA, where 100 g of BSE cattle brainstem was administered orally to 30 cattle as well as 100 g or 1 g to 100 cattle each (refer to III. 1. 1) a) Studies performed by the UK group for more details). In their study, vacuolar degeneration in the brainstem was observed at 32 months post-exposure in the 100 g dose group and at 66 months post-exposure in the 1 g dose group. PrP^{Sc} was detected first in the CNS at 30 months post-exposure in the 100 g dose group and at 44 months in the 1 g dose group. Before these time points, PrP^{Sc} was not detected in any nervous tissues examined. In addition, PrP^{Sc} was not detected in both of the cranial cervical and stellate ganglions[38].

Using highly sensitive WB, Masujin *et al*. investigated the period of PrP^{Sc} accumulation in tissues[15] of cattle administered orally with BSE cattle brainstem homogenate (of which infectious titer was estimated using RIII mice to be approximately $10^{3.1}$ i.c./i.p. ID_{50}/g) at a dose of 100 g or 1 g of the brainstem. Examined tissues were the brainstem, spinal cord, DRG, phrenic nerve, radial nerve, sciatic nerve, stellate ganglion, and adrenal gland of cattle at 27 to 42 months post-exposure for the 1 g dose group, and those of cattle at 36 to 51 months post-exposure for the 100 g dose group. In the 100 g group, clinical signs were observed first at 35 months, and PrP^{Sc} was detected first in the brainstem, cervical and thoracic spinal cords, and cervical DRG at 32 months, in the thoracic DRG at 35 months, in the phrenic nerve and adrenal gland at 35 to 36 months, and in the stellate ganglion and sciatic nerve at 36 months post-exposure. Clinical signs were observed first in an animal of the 1 g group at 44 months, where PrP^{Sc} was detected in the midbrain, cervical and thoracic spinal cords, thoracic DRG, and sciatic nerve. PrP^{Sc} was not detected in the peripheral nerve ganglion and adrenal gland of cattle that were PrP^{Sc} negative in the brain. PrP^{Sc} accumulations in the peripheral nerve ganglion and adrenal gland were observed at the same time as or later than detection of PrP^{Sc} in the brainstem. The presence of infectivity in PrP^{Sc} positive vagal nerves and adrenal glands was confirmed by i.c. inoculation of them to (BoPrP) Tg mice (supplied by Dr. Prusiner)[39].

2. Findings on Field BSE Cases

Buschmann *et al*. studied the infectivity of tissues of a field BSE case found in Germany with clinical symptoms at late-stage. For bioassay, RIII mice, Tga20 mice (over-expressing mouse PrP) and Tgbov XV mice were received i.c. and i.p. inoculation of a volume of 20 μL and 100 μL, respectively, of tissue homogenates or undiluted fluids including amniotic fluid, and then observed for 700 days. Tissues examined were the brainstem, thoracic and lumber spinal cords, retina, optic nerve, facial nerve, sciatic nerve, radial nerve, distal ileum, cerebrospinal fluid, spleen, tonsils, mesenteric lymph nodes, musculus semitendinosus, musculus longissimus dorsi, heart, caruncle, amniotic fluid, and colostrum. Among these tissues and fluid, infectivity was detected in the brainstem, thoracic and lumber spinal cords by RIII mice bioassay.

Infectivity was detected furthermore in the brainstem, spinal cord, retina, optic nerve, distal ileum, facial nerve, sciatic nerve, and musculus semitendinosus by bioassay using Tgbov XV mice that are more sensitive to the BSE agent than RIII mice. The infection rates in Tgbov XV mice (the number of onset mice / the number of inoculated mice) inoculated with the brain and thoracic and lumber spinal cords tissues were 100%, and the average survival times from inoculation were 208, 262, and 236 days, respectively. The infection rates after inoculation of the retina, optic nerve, facial nerve and sciatic nerve tissues were 10/13, 13/14, 11/14, and 9/13, respectively, with the average survival time of 331, 407, 526, and 438 days, respectively. These findings suggest that PrP^{Sc} accumulates in the peripheral nerve ganglion to lower extent than in the brainstem resulting in lower infectivity in the peripheral nerve ganglion than in the brainstem. The infection rate and average survival time detected with the distal ileum were 3/13 and 574 days, respectively. When the musculus semitendinosus tissue was inoculated into 10 mice, one mouse died at 520 days post-inoculation, thus the musculus semitendinosus was considered to be infectious. From these results, the authors concluded that only limited tissues of the BSE-infected case possessed infectivity. They also assumed that the infectivity of musculus semitendinosus was attributable to the distribution of sciatic nerves, and estimated the infectivity of the muscle to be $1/10^6$ of that of the brainstem[27].

The same group studied the accumulation of PrP^{Sc} and infectivity in various tissues derived from cattle experimentally infected with the BSE agent and from two natural cases found in the UK at terminal stages. The tissues examined were, the brainstem, optic and facial nerves, trigeminal and anterior cervical ganglions, medial/caudal cervical ganglion, nasal mucosa and tongue. A high level of PrP^{Sc} accumulation was detected only in the brainstem, while low levels of PrP^{Sc} accumulation were detected in the optic nerve and trigeminal ganglion, by SAF-immunoblot **[**scrapie associated fibril immunoblot: a method to detect PrP using a reaction of specific antibody with scrapie associated fibril (synonymous with PrP^{Sc}) transferred on nylon membrane.**]** and PMCA method **[**protein misfolding cycle amplification method: a method for amplification of PrP^{Sc} by ultrasonic wave treatment of PrP^{Sc} containing tissues in the presence of normal PrP.**]**. Bioassay using TgbovXV mice indicated the presence of infectivity in the tongue and nasal mucosa. However, their infection titers were $10^{2.5}$ ID_{50}/g or less, and PrP^{Sc} deposition was not detected in any of these tissues[40].

Using IHC and WB methods, Iwata *et al*. studied the distribution of PrP^{Sc} in various tissues in three BSE-positive cattle found at their age of 80 to 95 months in slaughterhouses in Japan. The examined tissues were the liver, spleen, kidney, heart, lungs, tongue, stomach, duodenum, distal ileum, small intestine at the position of two or six meters from the distal end, caecum, rectum, colon, retina, pancreas, adrenal cortex, lymph nodes, palatine tonsil, muscle, frontal lobe, caudate nucleus, optic thalamus, corpus striatum, hippocampus, occipital lobe, cerebellar cortex, medulla oblongata, DRG, and peripheral nerves, and cervical-, thoracic-, and lumber-spinal cords. None of clinical signs of BSE was observed in these three animals. PrP^{Sc} deposition was detected in the cerebellar cortex, medulla oblongata, and dorsal root ganglion, and cervical-, thoracic-, and lumber-spinal cords in all the animals. PrP^{Sc} was detected also in the femoral and lumber nerves (30 cm from the DRG), but with a trace amount estimated to be 1/1,000 to 1/4,000 of that detected in the spinal cord. In the distal ileum (PP), spleen, and lymph nodes including palatine tonsils, PrP^{Sc} was not detected[41].

Infectivity of tissues derived from two young BSE cases found in Japan was studied by Yokoyama *et al*. using TgBoPrP mice (supplied by Dr. Prusiner). One of the two was an atypical BSE case aged 23 months, the eighth case in Japan (BSE/JP8), and the other was a BSE case aged 21 months, the ninth case in Japan (BSE/JP9). None of clinical signs was observed in these cases. PrP^{Sc} deposition was not detected in the medulla oblongata of either animal by IHC. Although PrP^{Sc} could not be characterized in the tissue sample of BSE/JP8 by conventional WB, PrP^{Sc} was detected by WB method combined with phosphotungstic acid preparation (PTA) treatment for concentration of protein in the sample. The band pattern on WB was different from that of reported typical PrP^{Sc}, therefore judged as atypical BSE. For the sample of BSE/JP9, PrP^{Sc} was detected as a band of unglycosylated PrP^{Sc} after the peptide N-glycosidase F (PNGF) treatment to remove the sugar chain. From this result, BSE/JP9 was judged to be BSE positive.

The PrP^{Sc} accumulation in the tissues of the above two animals was very low, and estimated to be 1/1,000 of that in the brain of the sixth BSE case in Japan (BSE/JP6). To examine infectivity of the tissue from these two young cases, the authors intracerebrally inoculated the brain homogenate of BSE/JP8 and JP9 to TgBoPrP mice, and subsequently inoculated the brain of these TgBoPrP mice to other TgBoPrP and ICR mice to examine over two generations. No infectivity was detected throughout the bioassay. The sensitivity of this bioassay was equivalent to infectious titer of $10^{2.7}$ i.c. TgBoPrP mouse ID_{50}/g[3,4].

Okada *et al*. examined PrP^{Sc} in the intestinal tissues of four field BSE cases, those were found at their age of 54, 64, 69, and 102 months in the fallen stock surveillance program in Japan, by using highly sensitive IHC with alkaline treatment and WB with PTA treatment (PTA-WB). PrP^{Sc} was detected in the jejunum and ileum containing CPP, at the position of one meter or 30 centimeters from the ileocecal junction. PrP^{Sc} was also detected in the colon of the case aged 54 months. However, PrP^{Sc} was not detected in the duodenum, jejunum with and without DPP, ileocecal junction, caecum, and rectum of any cases. Infectivity of the distal ileum and colon of the case aged 54 months was examined by i.c. inoculation of the tissue homogenates into TgBoPrP mice. Since periods from inoculation of the distal ilem and colon to the death of mice were 528.7 ± 10.2 and 421.7 ± 48.2 days, respectively, the authors considered the infectivity of these tissues to be lower than that of the brain[42].

The same authors studied the immunohistochemical pattern and distribution of PrP^{Sc} in the CNS (brain and spinal cord) of seven field BSE cattle found as PrP^{Sc} positive cases in the fallen stock surveillance program in Japan at their ages between 54 and 89 months. Although none of the seven presented clinical signs, the PrP^{Sc} accumulation was observed widely in the entire areas of their brain and spinal cord. The accumulation was more intensive in the thalamus and brainstem than in the cerebral cortices and hippocampus[43].

3. Summary of Scientific Findings from Laboratory Studies

1) Relationship between the oral administration dose of BSE prions, incubation period, and incidence rate

According to Wells *et al*. (2007), the oral dose and incubation period showed an inverse relation in experimentally infected BSE cattle, as the smaller the dose was, the longer the mean incubation period. The incidence rate decreased as the dose decreased. Mean incubation periods, the periods from inoculation to clinical onset, were 31, 41, 45, and 53 months in the cattle dosed with 100 g, 10 g, 1 g, and 100 mg, respectively, of the brainstem from natural BSE cases. In cattle exposed to amounts less than 100 mg, the incidence rate was remarkably low and the incubation period deviated from the standard curve.

These findings were obtained under limited experimental conditions and it remains unclear whether oral administration of brain homogenate as a method for exposing is comparable to feeding on MBM produced by heat treatment. Nonetheless, the findings provide valuable information to estimate a relationship between doses of exposure and incubation periods of field BSE cases.

2) Relationship between the oral administration dose of BSE prions and the timing of deposition of detectable PrP^{Sc} in the CNS

The timing of deposition of detectable PrP^{Sc} in the CNS of orally exposed BSE cattle were 24, 34, and 44 months after administration of homogenate each equivalent to 100 g, 5 g, and 1 g, respectively, of the brainstem from natural BSE cases. The smaller the dose is, the longer the period to detection of PrP^{Sc}. The earliest detection was at 30 months after administration of the homogenate equivalent to 5 g of the brainstem, and at 42 months after administration of the homogenate equivalent to 1 g of the brainstem. Another study with administration of the homogenate equivalent to 100 g of BSE-affected brain has reported the presence of infectivity in the thoracic spinal cord and others earlier than in the medulla oblongata at the level of obex. However, PrP^{Sc} was not detected by IHC, and thus the accumulation of PrP^{Sc} in these tissues was judged to be very low. In contrast, a study of experimental BSE cattle in Japan has shown that oral inoculation of the homogenate equivalent to 5 g of brain tissue did not cause detectable deposit of PrP^{Sc} in the medulla oblongata at the level of obex at 48 mpi, although PrP^{Sc} was detected in the spinal cord by IHC.

Among BSE cases identified by inspection on dead cattle at the age of 24 months and over in Japan, the youngest animal was of age 48 months (born in October 2000). On the other hand, the youngest BSE case identified by inspection on cattle for human consumption was of age 57 months (born in August 2000) except two animals aged 21 and 23 months. In the BSE case of age 21 months (BSE/JP9), the PrP^{Sc} accumulation in the medulla oblongata at the level of obex was estimated to be approximately 1/1,000 of that in a classical BSE case. The infectivity was not observed in tissues of this atypical BSE case even by bioassay using Tg mice over-expressing bovine PrP that are highly susceptive to BSE prions.

3) Estimation of intake and incubation period based on studies of oral administration of BSE prions

The average incubation period (the period from the intake of BSE prions to the onset) during the days of a high incidence of naturally occurred BSE in the UK has been estimated to be 5 to 5.5 years. Based on the estimated incubation period of naturally affected cases and the incubation period observed in the experimental BSE cases, the amount of BSE prions fed by cattle in the field conditions in the UK have been estimated to be equivalent to the amount contained in 100 mg to 1 g of BSE-affected cattle brain used in the oral infection studies.

4) Presence of BSE prions in tissues other than SRMs of BSE cattle

The infectivity or PrP^{Sc} was detected in the adrenal gland and peripheral nerves besides the SRMs in both experimentally and naturally infected BSE cattle, but the timing of detection in these tissues was the same as or later than that in the CNS, suggesting that a large part of PrP^{Sc} or prion infectivity in the adrenal gland and peripheral nerve spreads differently from the CNS.

5) Presence of BSE prions in the intestine of BSE cattle

Although locations of PrP^{Sc} deposits in the intestine vary with reports, the distribution of PrP^{Sc} and prion infectivity in the intestine have been found mainly in the distal ileum of both experimental and natural BSE cases. PrP^{Sc} was detected in the ileum first at four months after oral administration with the homogenate equivalent to 100 g of the brain of field BSE cases. In addition, the infectivity and PrP^{Sc} accumulation were detected in the jejunum, but very low rates of infection in mice suggested that the infectivity titer was remarkably low. Oral administration with the tissue homogenate equivalent to 5 g of the brain of field BSE cases induced detectable deposit of PrP^{Sc} in a part of the ileum including distal ileum (two meters or more from the ileo-cecal junction). However, the amount of accumulated PrP^{Sc} was considered to be small, because the frequency of PrP^{Sc} positive lymph follicles was very low. Taken together with the findings of oral infection studies in the UK with 100 g and 1 g administration, the result of 5 g administration suggests that the PrP^{Sc} accumulation in ileum may decrease and take place at more distal locations with the decrease in dose. In addition, the PrP^{Sc} accumulation was hardly detected in the intestine of cattle received 1 g administration.

Moreover, PrP^{Sc} deposition and prion infectivity were detected in the distal ileum of field BSE cases, although the infectivity titer was low. Hence, BSE prions are considered to be present in the distal ileum for a long period after infection.

IV. BSE Infection in Cattle Population

1. Japan

1) Overview of feed control measures

a) Import of live cattle, MBM, and others

Japan banned the import of live cattle from the UK in 1990. Subsequently the import from BSE countries has been suspended. Since 2001, the import from all EU-countries has been prohibited regardless of the prevalence of BSE cattle in each country. The import from non-EU countries will be suspended immediately when BSE cases are detected. The import of livestock requires a certificate of animal health (animal health requirement) agreed between the government agencies of the exporting countries and the Ministry of Agriculture, Forest and Fishery of Japan (MAFF).

Since October 2001, the government has prohibited the import of animal-processed protein, and animal oil/fat, as well as feed and manure produced from these protein and fat sources, as control measures for MBM and animal oil/fat.

When the government agencies of the exporting countries prove that MBMs and animal oil/fat meet the requirements such as those of pig-origin, these materials are exempt from import ban. However, all MBMs, meat meal, and bone meal imported into Japan are the subject of inspection at the Animal Quarantine Service upon arrival based on the Act on Domestic Animal Infectious Diseases Control (Act No. 166 of 1951). Fish meal is exempt from import ban when the government agency of the exporting country proves that it does not contain animal processed proteins other than fish meal, but samples are thoroughly inspected in order to confirm that it is free from other animal-processed proteins. If contamination is confirmed in fish meal, import from the fish meal processing plant is stopped.

All import applications are the subject of thorough inspection for the concentration of insoluble impurities in animal oil/fat which is used or may be used for feed being below 0.15%[11,44].

b) Feed control measures

In April 1996, an administrative guidance was issued by MAFF to impose a voluntary ban on the use of ruminant MBM for ruminant feed. In September 2001, the ministry prohibited the use of ruminant proteins (excluding milk, milk products, gelatin, and collagen) for ruminant feed based on the Ministerial Ordinance on Standards of Ingredients of Feed and Feed Additives (MAFF Ordinance No. 35 of 1976). In October of the same year, the ministry prohibited the use of all animal-derived proteins for ruminant feed and the use of ruminant-derived proteins for non-ruminant.

Moreover, the import of ruminant MBM for feed ingredients was banned from all countries and regions. MBMs produced in Japan are incinerated, and hence, ruminant MBMs are not domestically distributed. In addition, preventive measures against cross-contamination are adopted in slaughterhouses, rendering facilities, and feed plants[11,44].

2) BSE surveillance

In 1996, MAFF designated BSE as a notifiable disease in the Act on Domestic Animal Infectious Diseases Control. MAFF started BSE testing on fallen cattle suspected of being infected with unidentified diseases, including those sent to the Livestock Hygiene Service Centers. In April 2001, following the recommendations of the OIE, the ministry included cattle showing signs of CNS impairment in the subject of BSE inspection, and has tested all fallen cattle aged 24 months or older since April 2003. MHLW started BSE testing on cattle of all ages at slaughterhouses since October 2001. Subsequently, based on the risk assessments conducted by FSCJ in August 2005, the MHLW changed the age of cattle to be tested for BSE to 21 months or older. However, all local governments (including cities designated by Community Health Act) have continued to conduct voluntary BSE test on cattle aged less than 21 months. Screening by ELISA test, a rapid test for BSE, on samples of medulla oblongata at the level of obex has also been included in the voluntary BSE test.

When the rapid BSE test indicates a positive result on the obex sample from the risk cattle including fallen cattle, the sample is served to confirmatory tests with WB and IHC. If one or both of WB and IHC exhibit a positive result, the animal is judged as a case of BSE **[**Definitive confirmation is provided based on opinions of the Prion Subcommittee when needed.**]**. When the rapid BSE test on the obex sample from cattle slaughtered at slaughterhouses indicates a positive result, the sample is also served to confirmatory tests with WB and IHC, and if one of them exhibits positive result, the Expert Committee is asked for its opinion prior to the final diagnosis[11,45–48].

3) BSE prevalence

a) Overview of BSE prevalence

The total number of BSE cases identified in Japan is 36. The number of the cases increased from three in 2001 to 8 in both 2005 and 2006, but the number decreased subsequently to three in 2007 and one in 2008. BSE cattle have not been reported since the case of fallen cattle aged 101 months detected in January 2009 (as of July 2012).

With an exception of one case found in Chiba prefecture in September 2001, BSE tests have been conducted on 12,852,252 cattle (as of March 2012, BSE screening test result from the website of MHLW: http://www.mhlw.go.jp/houdou/0110/h1018-6.html.) at slaughterhouses, and among them 21 cases were confirmed to be BSE positive. Two of the 21 cases were detected in cattle aged less than 30 months of age: cattle aged 21 months (born in January 2002 and detected in November 2003) and aged 23 months (born in October 2001 and detected in October 2003). The latter was classified as atypical BSE by WB. In addition to this case, another atypical BSE case was detected in March 2006 at the age of 169 months, and thus totally two atypical BSE cases have been found in Japan. With the exception of the above two BSE cattle aged less than 30 months, the range of cattle age at BSE confirmation was from 57 to 185 months, the average age being 88.0 months.

The surveillance program for fallen cattle identified 14 cases among 834,349 (the total number of cattle tested as of March 2012, The website of MAFF: http://www.maff.go.jp/j/syouan/douei/bse/b_sarvei/index.html). Age of cattle at the time of BSE confirmation ranged from 48 to 102 months, and was 75.7 months in average.

Cattle showing typical clinical signs of BSE have not been found by the surveillance program in Japan[2].

Table 6 shows the number of cattle tested for BSE and the number of BSE positive cattle.

b) Characteristics of birth cohort

Fig. 5 shows the number of classical BSE cases in Japan by calendar year of birth, and **Table 7** shows a profile of a BSE case born after the feed control measures were tightened.

Fig 5 shows that the first-born case was found in cattle born in 1992, aged 185 months at the time of confirmation in 2007. Subsequently, relatively high incidence was seen in two birth cohorts (the birth cohort means cattle population of the same birth year): 12 cases in the birth cohort of 1996 and 13 in that of 2000. No BSE cattle have been found in the birth cohorts after February 2002 (as of September 2012).

Table 6. The annual number of cases tested for BSE, the number of confirmed BSE cases and the age range (months) at the time of detection

Fiscal year	Number of BSE tested		Number of BSE cases per year*	Age (months) at the time of detection				
	Healthy slaughter	Risk cattle including fallen stock		<21	21–30	31–48	49–72	>72
2001	523,591	1,095	3 (2)		2(2)	1(0)	3 (2)	4 (4)
2002	1,253,811	4,315	4 (4)				1 (1)	2 (1)
2003	1,252,630	48,416	4 (3)				6 (3)	3 (2)
2004	1,265,620	98,656	5 (3)				5 (2)	2 (2)
2005	1,232,252	95,244	8 (5)					3 (1)
2006	1,218,285	94,749	8 (3)					3 (1)
2007	1,228,256	90,802	3 (1)					1 (0)
2008	1,241,752	94,452	1 (0)					
2009	1,232,496	96,424	0					
2010	1,216,519	105,380	0					
2011	1,187,040	104,816	0					
Total	12,852,252	834,349	36 (21)		2 (2)	1 (0)	15 (8)	18 (11)

* Numbers in parentheses refer to the number of confirmed BSE cases at slaughterhouses (21 cases). In Japan, 36 cattle have been confirmed to be BSE cases and the first case was confirmed in Chiba prefecture in September 2001.

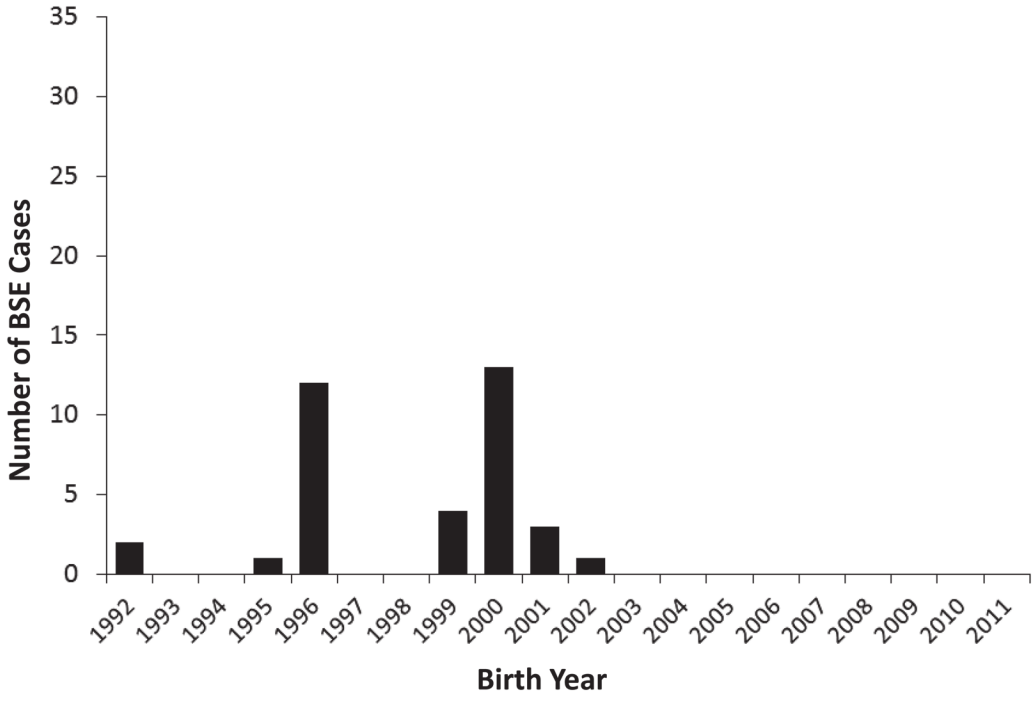


Fig. 5. Year of birth distribution of BSE cases in Japan

Table 7. Profile of a BSE infected cow born after the feed ban was implemented

Date of birth	Year of diagnosis	Age (Months)	Surveillance stream
Jan. 2002	2003	21	Healthy slaughtered

The latest-born BSE case was a Holstein-Friesian steer (BSE/JP9) born in January 2002, which was diagnosed as BSE at the age of 21 months. This cow was born after the reinforced feed ban in October 2001, and is considered to be exposed to remaining feed sold before the measures were reinforced, because it was not withdrawn from the market[44]. The accumulation level of PrP^{Sc} in the medulla oblongata at the level of obex of this case was estimated to be approximately 1/1,000 that of the BSE case confirmed at 83 months of age (BSE/JP6) **[**The young cow was diagnosed as BSE in the surveillance. WB, IHC, and histological examinations indicated BSE positive.**]**. No infectivity was observed in the brainstem of this young case by bioassays using Tg mice overexpressing bovine PrP (TgBovPrP) and ICR mice intracerebrally inoculated with the brainstem homogenate **[**The remains of samples used for ELISA were used for the bioassays due to the shortage of samples.**]**. The results of the bioassays suggest that the infectivity in the brain of this case, if any, is very low[3]. The young age indicates that a relatively large amount of feed containing ruminant proteins might were fed to this animal. The question arises, if calves were exposed to large amounts of BSE agent around 2002, there should have been other BSE cases detected among birth cohorts around 2002. However, no BSE positive cattle were found in the birth cohorts of 2002 and 2003 except for two cases, and only two BSE positive cattle were found in the birth cohort of 2001. Thus, the number of BSE positive cattle in these birth cohorts were remarkably low compared to 13 cases in the birth cohort of 2000[44,49].

The cattle in the birth cohort of 1996 were already five years of age in 2001 when surveillance at slaughterhouses started, and around eight years of age in April 2004 when the full implementation of surveillance for fallen cattle aged 24 months or older started. The BSE status in Japan was estimated using recorded BSE cases born in 1995 and 1996 in the risk assessment on "Food Safety Risk Assessment Related to Measures against Bovine Spongiform Encephalopathy (BSE) in Japan"[16], although limited numbers of cattle were tested for these birth cohorts. As for the birth cohort of 2000, the number of confirmed BSE cases peaked at their age of five years, and the age at confirmation ranged from 48 to 101 months with the average of 70.5 months.

2. The US

1) Overview of feed control measures

a) Import of live cattle, MBM and others

The US banned the import of live cattle from the UK in July 1987, and subsequently, banned the import from BSE risk countries (Europe in 1997, Japan in 2001, and Canada in 2003). In 2005, the import from BSE minimal-risk countries **[**Non-BSE countries, BSE countries that the US judges to be of low risk, etc.**]** was reopened, and from Canada, cattle aged less than 30 months for slaughtering including fattening cattle were allowed for import. In November 2007, the US expanded allowable import of live cattle born on or after 1 March 1999 from Canada for any use. These cattle were born after feed control measures taken by the government came into effect[50–54].

The US has prohibited the import of MBMs from the UK since November 1989, and subsequently the import of MBMs which cannot be identified to be derived from non-ruminants has been prohibited from BSE occurring countries (Europe in 1997, Japan in 2001, and Canada in 2003). The US government banned the import of all processed proteins of animal origin (excluding proteins confirmed to be derived from pigs, chickens, and fish meal) in December 2000[55] and the import of tallow of animal origin (excluding those for industrial use and linolenic, stearic, and glycerin acids derived from tallow) in December 2000, from the BSE risk countries designated by the US. In January 2005, the import of tallow composed of less than 0.15% insoluble impurities from BSE minimal-risk country (Canada) was resumed[53].

b) Feed control measures

The US banned the import of MBM from BSE occurring countries and the use of mammal proteins for feeding ruminant animals in 1989 and 1997, respectively. However, the followings are excluded from the prohibited mammal proteins: milk and milk products, blood, blood products, gelatin, proteins derived from pigs and horses, inspected meat products which have been cooked and offered for human food and heat processed for feed (such as plate waste)[13].

Moreover, the feed regulation was enhanced in October 2009, and the followings were included in Cattle Materials Prohibited in Animal Feed (CMPAF) and prohibited from the use in all animal feed and pet food: the entire carcass of BSE positive cattle; the brains and spinal cords of cattle aged 30 months of age and older; the entire carcass of cattle not inspected and passed for human consumption excluding that of cattle under 30 months of age or cattle from which the brains and spinal cords were removed; tallow derived from BSE positive cattle; tallow derived from CMPAF that contains more than 0.15% insoluble impurities in weight; and mechanically-separated beef derived from CMPAF.

In addition, preventive measures against cross-contamination are in place in slaughterhouses, rendering facilities and feed plants[13,14].

2) BSE surveillance

Since May 1990, the US has taken measures to prevent the entrance of the BSE agent and potential spread of BSE, and has conducted BSE surveillance targeted at cattle aged 24 months or older with signs of CNS impairment or non-ambulatory status. Upon confirmation of the first BSE case in December 2003, the BSE status of US cattle has been monitored and assessed for two years from June 2004 to enhance surveillance program to determine the level of disease present in the US cattle population more accurately than ever[17]. In the enhanced surveillance, test targets were expanded to include healthy slaughter cattle. Over the time of the implementation period (approximately 22 months) of the enhanced surveillance, out of 670,000 samples of cattle tested, two cases of BSE were identified: On 24 June 2005, one US born case of estimated birth year of 1992, and on 13 March 2006, one US born case of estimated birth year of 1995 were confirmed. On April 2012, one BSE case of estimated birth year of 2001 was confirmed. These cases were confirmed to be atypical H-type BSE (H-BSE) cases[56,57]. Based on the analysis of surveillance data until March 2006, the prevalence of BSE among US cattle was estimated to be one in one million cattle. On the basis of the result, the current ongoing surveillance program was established in July 2006, and then BSE testing has been done targeting annually 40,000 high-risk cattle including cattle of all ages displaying BSE clinical signs and of non-ambulatory status (downer cattle) aged 30 months or older. The ongoing surveillance is designed to detect BSE at a prevalence level of one per one million cattle, and meets the surveillance program set by OIE to detect BSE at a prevalence of one case per 100,000 cattle[56,58].

Since 1990, the National Veterinary Service Laboratory (NVSL) has conducted surveillance tests by IHC method described in the OIE manual as well as diagnosis by WB. Since June 2004, veterinary authorities and diagnostic veterinary laboratories in seven states approved by NVSL[59] have used the ELISA screening test as well as the IHC and WB confirmatory tests. NVSL is engaged in a part of screening tests and all of confirmatory diagnosis on suspected cases[17,56,60].

Table 8 shows the number of cattle tested annually under the BSE surveillance program in the US.

3) BSE prevalence

a) Overview of BSE prevalence

Four BSE positive cattle have been confirmed as of July 2012 in the US. The first case was detected in Washington in December 2003, that was a dairy cow imported from Canada. The second and third cases were confirmed in a domestic beef cow in Texas in June 2005 and that in Alabama in March 2006, respectively. The fourth case was confirmed in a domestic dairy cow in California in April 2012. All the three domestic cattle were aged ten years or older and were reported to be atypical BSE cases[62–66].

b) Characteristics of birth cohort

Fig. 6 shows the number of BSE cases by year of birth. The latest–born case was a female Holstein-Friesian cow born in September 2001 and diagnosed as BSE at the age of 127 months.

3. Canada

1) Overview of feed control measures

a) Import of live cattle, MBM and others

Canada banned the import of live cattle from the UK and Ireland in 1990, from BSE occurring countries in 1994, and from countries not recognized as free from BSE by the Canadian Food Inspection Agency (CFIA) in 1996[67,68]. In April 1998, the government revised import policy and approved the import of ruminant animals from countries classified as free from BSE [Australia, Denmark, Finland, Iceland, New Zealand, Norway, Sweden, and the United States][67,69]. In December 2005, the government adopted BSE import policy where exporting countries were classified into three

Table 8. The annual number of cattle tested for BSE under the BSE surveillance program in the US

Year[*1]	Number of cattle tested for BSE				BSE positive[*2]
	Healthy slaughter	Fallen stock	Emergency slaughter	Clinical suspects	
1999	35	15	351	265	0
2000	24	0	2,063	664	0
2001	159	1	4,516	665	0
2002	948	2,818	16,045	569	0
2003	481	3,106	16,612	578	0[*3]
2004	1,869	62,071	25,095	1,066	0
2005	6	361,986	50,777	1,534	1
2006	19,904	272,778	20,703	1,416	1
2007	1	27,175	12,821	3,339	0
2008	0	26,479	14,224	2,442	0
2009	0	27,748	14,093	2,376	0
2010	0	28,827	13,099	2,375	0
2011	0	23,626	9,467	1,987	0

[*1] A term of each year: 1999, 1 April −30 September; from 2000, 1 October of the year before −30 September; 2011, 1 October 2010 − 31 August 2011.

[*2] The website of OIE, "Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide" **[**The website of OIE: http://www.oie.int/?id=505.**]** .

[*3] Cattle imported from Canada where BSE was confirmed in 2003 are excluded from the number of BSE cattle in the US.

Based on consultative reference of the US[61)]

categories: negligible BSE risk, controlled BSE risk, and undetermined BSE risk. Currently, the import policy adheres to the above OIE categories[70,71)].

Following the BSE case identified in the US in December 2003, Canada implemented a restriction on the import of US live cattle excluding those imported for immediate slaughter[72)]. The import of feeder calves (male calves) and cattle for temporary stay in Canada was resumed in April 2004[73)]. The import of cattle under 30 months of age was resumed in March 2005[74)]. In June 2006, the import of all the cattle born in the US after 1999 was approved[75)]. The import of meat meal, bone meal, and blood meal was banned from all countries except the US in 1988[67,76)]. In 1996, the following import was banned from countries not recognized by Canada as free from BSE: animal and pet food or material imported as ingredients of animal and pet food containing materials of ruminant origin[77)].

All rendered animal products became under the restriction of use for ruminant feeds in 1997, and this restriction was applied also to permission of their import. Moreover, the import of rendered products of ruminant origin except blood and milk were banned from countries not recognized as free from BSE. In 1998, the products derived from sheep and goats were included in the import policy[78)]. The exporting country was required to certify that the animal had been slaughtered in that country.

In 2000, Canada suspended the import of animal protein products including blood and feather meal from any species from any countries that Canada does not designate as a country free from BSE (except for rendered blood products for use in farmed fish from France and MBM of pig-origin for use in farmed fish from Denmark)[79)]. The import of inedible animal oils and fats from the US started in 1982[80)]. In 1988, the import of all animal oil and fat was approved from the US. In 1996, tallow was excluded from the import regulations specific to BSE, and approved for import for any use from Australia, Denmark, Finland, Iceland, New Zealand, Norway, and Sweden[77)]. In 2000, protein-free tallow and products derived from tallow were approved for import from countries not designated free from BSE, if these imports were certified to be protein free with the maximum level of insoluble impurities of 0.15% by weight, and preventive measures against cross-contamination were certified to be in place.

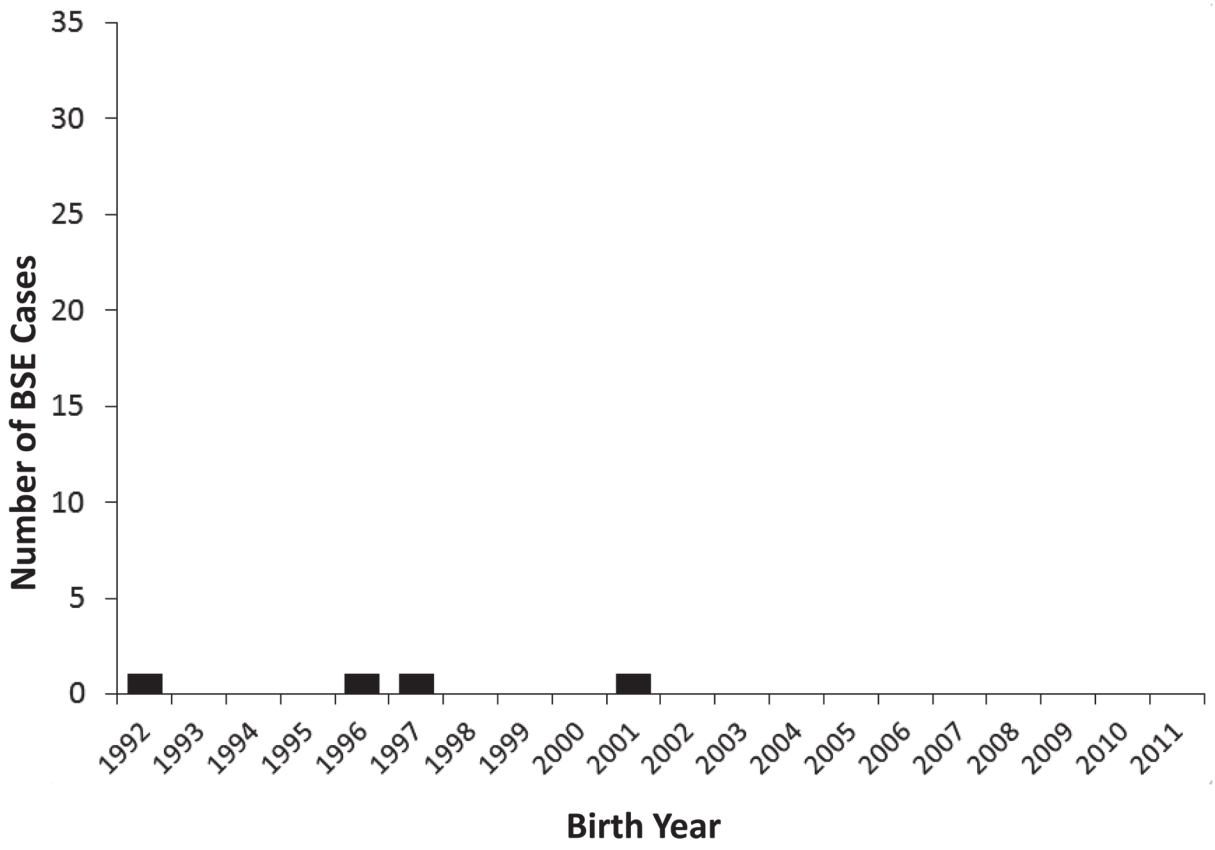


Fig. 6. Number of BSE cases in the United States by year of birth
Note 1) The exact birth years of the first three cases were not disclosed. (Birth years were estimated based on approximate ages of the cattle at the time of BSE confirmation).
Note 2) A cow imported from Canada and confirmed as a BSE case in the United States (born in 1997) was included.

In December 2005, the import policy was adopted to classify exporting countries into three categories (negligible BSE risk, controlled BSE risk, and undetermined BSE risk). The policy was amended in August 2010, and has been in place until today[71].

b) Feed control measures

In 1997, mammal protein derived from all mammals were principally banned from use in ruminant feed (materials banned from use in ruminant feed in Canada are hereinafter referred to as "prohibited materials")[67]. However, milk, dairy products, blood, blood products, gelatin, and proteins derived from pigs and horses were excluded from prohibited materials.

In July 2007, feed control measures were strengthened to ban the use of SRMs (the skull, brain, trigeminal ganglia, eyes, tonsils, spinal cord, and DRG of cattle aged 30 months or older and the distal ileum of cattle of all ages)[81] in all animal feed, pet foods, and fertilizers[82]. Concurrently, ruminant oil/fat containing more than 0.15% of insoluble impurities by weight was banned from feeding ruminants. Gelatin derived exclusively from (ruminant) skin was excluded from the ban on feeding ruminants. Moreover, the ban on feeding ruminant oil/fat containing more than 0.15% of insoluble impurities to ruminants mentioned above was expanded to include all animals.

Preventive measures against cross-contamination are in place at slaughterhouses, rendering facilities and feed plants[12,81–83].

2) BSE surveillance

In 1992, Canada started the surveillance of cattle showing signs of the CNS impairment and non-ambulatory status.

Following the first confirmation of domestic BSE cattle in Canada in May 2003, an expanded surveillance designed to estimate BSE prevalence in adult cattle population started in 2004. The surveillance was initiated to test 8,000 cattle in 2004 to increase the number to 30,000 annually after 2005[84].

The surveillance program started in 1992. According to the program, pathology laboratories of federal and provincial governments and universities have conducted histopathological screening of cattle showing symptoms of the CNS impairment. These cattle displaying clinical signs of BSE were sent from farms, and provincial and federal slaughterhouses[85].

In 2002, the surveillance program was expanded to include following categories of cattle: dead on arrival (DOA), emergency slaughter, and downers. In the same year, a large number of fallen cattle were also included in the surveillance program for testing[85].

The BSE surveillance program implemented in 2004 was designed to detect at least one BSE case at a confidence level of 95% when the BSE prevalence is two cases per one million adult cattle population. The surveillance scheme was planned to survey 8,000 cattle in the first year (2004), and 30,000 or more in each year from 2005[84,86]. The data from 2004 to 2008 were based on the OIE point system[87] **[**OIE codes require more than 0.3 and 0.15 million points in the past seven years to detect one BSE case in 0.1 and 0.05 million, respectively, with a confidence level of 95% when the number of adult fed cattle aged 24 months or older exceeds one million.**]**, and the surveillance program meets the level of OIE point system for detection of at least one case per 100,000 adult cattle.

Currently, rapid tests using ELISA etc are conducted within the Transmissible Spongiform Encephalopathy (TSE) Laboratory network, which includes provincial pathology laboratories and six facilities in the CFIA network laboratories. BSE positive samples are sent to the National BSE Reference Laboratory at the Canada's National Centre for Foreign Animal Disease for confirmatory diagnosis with IHC. If the brainstem (obex) cannot be identified due to sample conditions, or if there is a discrepancy in results between the rapid tests and IHC, WB is used for confirmation[86,88].

Table 9 shows the annual number of cattle tested for BSE under the BSE surveillance program in Canada.

3) BSE prevalence

a) Overview of BSE prevalence

In 1993, Canada's first BSE-positive case was confirmed in Salers cattle imported from the UK. In May 2003, the first domestic case of BSE was confirmed in Canada. As of July 2012, BSE has been confirmed in a total of 18 domestic cattle, two of which were characterized as atypical BSE (H and L types). Among the 16 cases of classical BSE, the youngest case was found in cattle aged 50 months and the oldest case was found in cattle aged 97 months, and the average age at confirmation was 75.8 months (6.3 years) **[**When age-in-month is uncertain, birth months were assumed to be the youngest age estimated.**]**. The two atypical BSE cases were confirmed at their age of ten years or more[90].

b) Characteristics of birth cohort

Fig. 7 shows the distribution of birth year of BSE cases in Canada. The latest born case was a female Holstein- Friesian cow born in August 2004, which was diagnosed with BSE at the age of 77 months.

4. France

1) Overview of feed control measures

a) Import of live cattle, MBM and others

The EC, covering the specific legislation areas of EU, banned the export of cattle born in the UK before 18 July 1988, and offspring of affected or suspect animals to all EU member states in July 1989[9,91]. In 1996, the EC banned the export of live cattle from the UK[9,92], and in 1998 banned the export of live cattle from Portugal to all EU member states. In 2004, the EC lifted the ban on export from Portugal, and in 2006 from the UK with imposing certain conditions[9,93,94].

In 1996, France introduced its own regulation to ban the import of live cattle from Switzerland and lifted the ban in 2002[9].

Since 2001, Annex IX to the TSE Regulation has laid down the specific trade rules covering imports of live bovine animals. The import requirements vary depending on the classification of the BSE risk status of the countries concerned. Council Directive 1979/542/EEC sets a list of third countries **[**Canada, Switzerland, Chile, Greenland, Croatia, Iceland, Montenegro, Macedonia, New Zealand, Saint Pierre, Miquelon, and Serbia (as of March 2009)**]**, from which imports are authorized. The health certification for importation is issued after the inspection at boarder inspection posts (BIP). These certificates became a requirement for transportation of approved live cattle within the EU member states[95,96].

France introduced its own regulation regarding the import of MBMs from the EU, and banned the import of blood meal, flours and meals for meat, offal, bones, and animal fat meals from the UK in August 1989, as well as from Ireland in December the same year (the ban on import from Ireland was lifted in 1993). In the regulation, an exceptional measure

Table 9. Annual number of cattle tested for BSE under the surveillance program in Canada

Year	Number of cattle tested for BSE		BSE positive[*2]
	Number of cattle tested[*1]	Clinical suspect	
1992	225	-	0
1993	645	54	1
1994	426	51	0
1995	269	67	0
1996	454	157	0
1997	759	244	0
1998	940	137	0
1999	895	692	0
2000	1,020	452	0
2001	1,581	623	0
2002	3,377	451	0
2003	5,727	286	2[*3]
2004	23,550	-	1
2005	57,768	-	1
2006	55,420	-	5
2007	58,177	-	3
2008	48,808	-	4
2009	34,618	-	1
2010	35,655	-	1
2011	33,458	-	1

[*1] Data after 2004 are based on the testing results on the website of CFIA

[The website of CFIA: http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/bse/enhanced-surveillance/eng/1323992647051/1323992718670.**]**.

[*2] The website of OIE, "Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide" **[**The website of OIE: http://www.oie.int/?id=505.**]**.

[*3] One of which was detected in Canada.

Based on the results of surveillance in Canada[84,89].

was adopted to allow import of meals derived from pig and ruminant animals, adding additional conditions such as the prohibition on the use of the imports for ruminant feed. However, this exceptional measure was withdrawn in February 1990[97].

In 1996, the export of mammalian MBMs from the UK to all EU member states was banned[92]. In 1998, the export of mammalian MBMs from Portugal to all EU member states was banned[93]. From 2002, documents such as health certificates issued by the competent authorities of final destination countries have been required to transport products classified as category 1 (including SRMs) and 2 (including MBMs) in the Animal By-products Regulation (2002/1774/EC)[98].

In March 2011, the Animal By-products Regulation (2009/1069/EC) was amended and its rules were made clear by prescribing the following issues: For the transportation of category 1 and category 2 materials, information shall be provided to the competent authorities of the origin and the destination; within a certain period of time, the competent

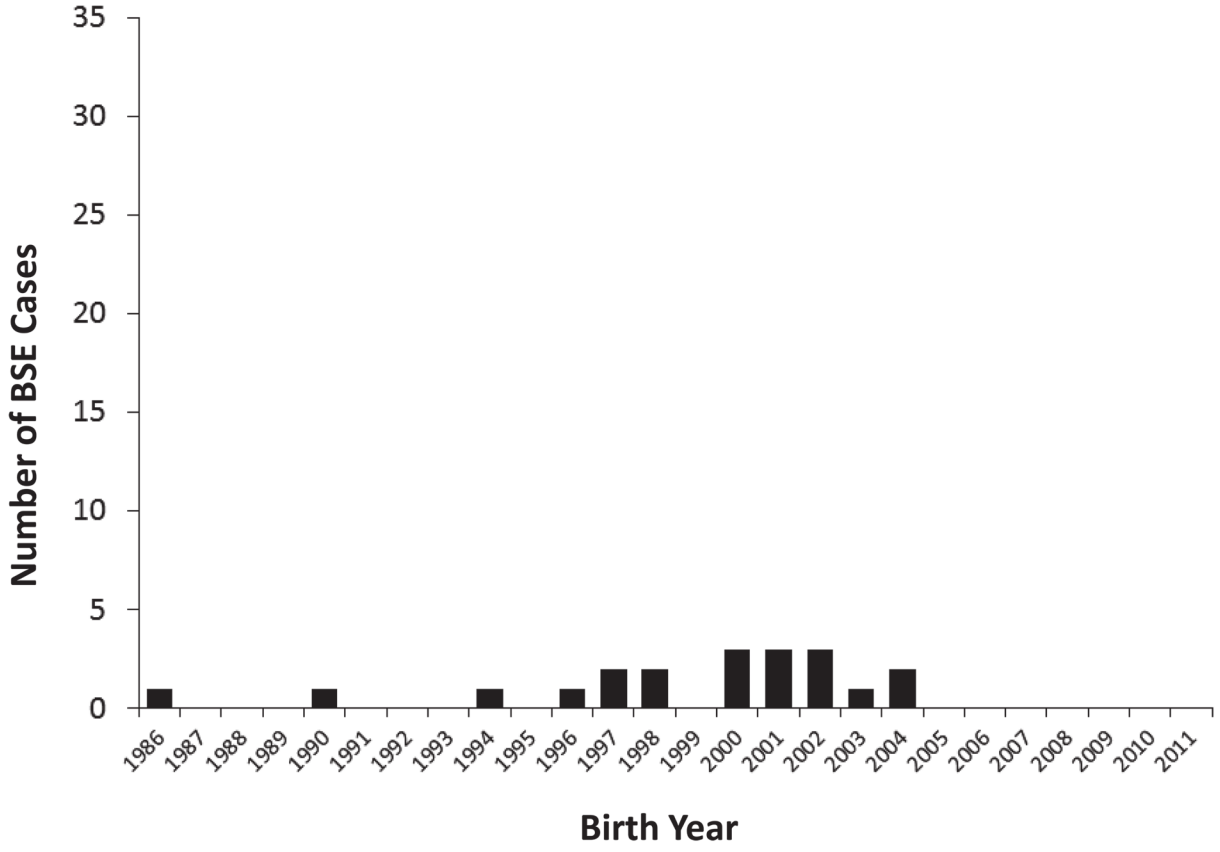


Fig. 7. Year of birth distribution of BSE cases in Canada
Note) A cow imported from the UK (born in 1996) and a cow imported from Canada and confirmed with BSE in the United States (born in 1997) were included.

authority of the destination shall decide acceptance of imports ; and introduction of items concerning transportation of consignments among EU countries via third countries.

b) Feed control measures

According to the explanation by the French government, it banned the import of all mammal proteins from the UK and their use for ruminants in 1989. The use of mammal-derived protein in feed for cattle and ruminants was banned in 1990[9)] and 1994[9,100)], respectively. In November 2000, the feeding of all mammal-derived proteins to all livestock was banned[9,101,102)].

After the enforcement of TSE regulations in 2001, animal proteins (excluding milk, dairy, and some others) and ruminant-derived oil/fat containing more than 0.15% of insoluble impurities were banned from use in all livestock feed[9)]. The fish meal is also prohibited from use in ruminants in France. In addition, preventive measures against cross-contamination are taken in slaughterhouses, rendering facilities and feed plants[9)].

2) BSE surveillance

In June 1990, France designated BSE as a notifiable disease, and started passive surveillance, i.e. surveillance through examination and mandatory reporting of animals showing clinical signs of BSE. Farmers must call the veterinarian whenever neurological signs appear on a bovine. The veterinarian must report the case to the Directions départementales des Services Vétérinaires (DDVS) if BSE is suspected. Failure to report such cases is a subject to punishment[9)].

The surveillance of fallen cattle started as a research program in 2000 and systemized in 2001 after 56,000 cattle were tested. Dead cattle aged over 24 months found in farms are sent to rendering plants to take samples for systematic test[9)].

BSE testing on healthy slaughter cattle aged over 30 months started in January 2001, and was conducted for those over 24 months of age from July the same year, and for those over 30 months of age again from July 2004 to December 2008. On 1 January 2009, the age limit for the BSE testing for healthy slaughter cattle was raised to 48 months as pro-

vided in European Commission Decision (2008/908/EC)[7], and on 1 July 1 2011, to 72 months as provided in European Commission Decision (2011/358/EC)[101,103].

From 1997, cattle confirmed to be BSE positive by histological examination were designated as subjects to be killed by euthanasia with all the relevant herds, and their corpses were to be incinerated. In 2002, this measure was brought down with amendment, and only the cohort cattle [The term, cohort cattle, herein refers to 1) cattle born 12 months before or after the birth of BSE positive cattle, 2) cattle raised with and fed with the same feed as BSE positive cattle during the first year after birth, 3) cattle born from female BSE positive cattle within two years before the BSE positive cattle died or presented symptoms.] became subjects to be killed by euthanasia followed by a sampling for BSE test[9].

Until 2002, all samples of cattle showing clinical signs were sent to the Agence Française de Sécurité Sanitaire des Aliments (AFSSA) [on 1 July 2010, AFSSA and gence Francaise de Securite Sanitaire de l'Environnement et du Travail (AFSSET) were merged into Adnimistracion Nacional de la Seguridad Social (ANSES).] and tested by IHC method. Rapid tests on healthy slaughter cattle started in January 2001, and those on dead cattle in June the same year. In 2002, rapid tests started also for cattle showing clinical signs.

Rapid tests are conducted in inspection facilities authorized by La Direction Générale de l'Alimentation (DGAL), and if the test results are not negative, samples are sent to AFSSA, where these samples are tested not only by rapid tests but also by WB and IHC for several regions of the brainstem to make a conclusive diagnosis[9].

Table 10 shows the annual number of cattle tested under the surveillance program in France. During the inspection year of 2011 (from 1 November 2010 to 31 October 2011), BSE test was conducted on 1,722,012 cattle including 1,414,857 healthy slaughter cattle, 289,385 dead cattle, 17,764 emergency slaughter cattle and 6 cattle showing clinical signs. Based on this surveillance data, the "point value" provided in the OIE terrestrial animal health code[104] was 312,138 in 2011, and meets the level of the OIE point system for detection of BSE at least one per 100,000 adult cattle[99,101].

3) BSE prevalence

a) Overview of BSE prevalence

The data reported to OIE show that after the first BSE positive cow was found in France in 1991, the number of BSE positive cattle peaked at 274 in 2001 and subsequently decreased to 239 in 2002, 137 in 2003, 54 in 2004, less than 10 between 2006 and 2008, 10 in 2009, 5 in 2010, 3 in 2011, and 1 in April 2012. The total number is 1,023 (as of July 2012, The website of OIE: http://www.oie.int/?id=505).

Among the 1,023 BSE positive cattle, the youngest case was aged 43 months, the oldest 227 months, and the average age was 86 months (7.1 years). Until December 2010, atypical BSE was found in 27 cattle, 14 of which were H-type and 13 were L-type [refer to the section VI Atypical BSE.][9,105–109].

b) Characteristics of birth cohort

Fig. 8 shows the distribution of the year of birth of BSE cases in France and **Table 11** shows details of BSE cases detected in cattle born after feed control measures were implemented.

The largest number of BSE positive cattle was born in 1995. Feed ban was fully implemented in France by November 2000 (banned the use of mammal proteins in feed for all livestock). The number of BSE positive cattle born after this enforcement of the feed ban (13ARB cases) was only three: a cow born in April 2004, the latest born case, and two cattle born in 2001.

The BSE case detected in 2004 was born three years after the start of the reinforced feed ban. While there is no direct evidence indicating the process of ingestion of the BSE agent by this BARB (born after the reinforced ban) case, the possibility cannot be excluded that feed contaminated with MBM remained inside the pipes in animal feed processing plants or at the bottom of silos after production of MBM for livestock other than cattle. AFSSA indicated the complexity of the flow of manufacturing, distribution, and the use of animal feed, and recommended to maintain the quality of active surveillance and the control measures for ruminant feed[110].

5. The Netherlands

1) Overview of feed control measures

a) Import of live cattle, MBM and others

EC banned the export of cattle born in the UK before 18 July 1988, and offspring of affected or suspect animals to EU member states in July 1989[111]. In 1996, the EC banned the export of live cattle from the UK[112] and in 1998 banned

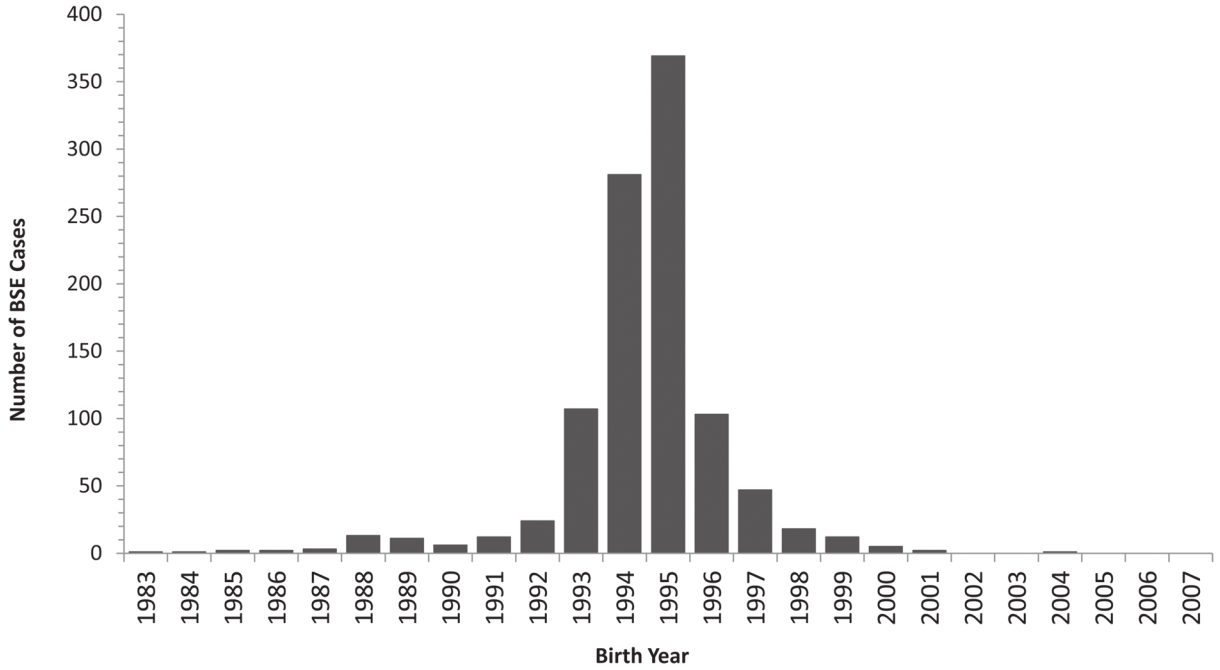


Fig. 8. Year of birth of BSE cases in France

Table 10. Annual number of cattle tested for BSE in France

Year	Number of cattle tested for BSE				BSE positive[*2]
	Healthy slaughter	Fallen stock	Emergency slaughter	Clinical suspect	
2001	2,351,396	122,775	—	91	274
2002	2,889,806	271,520	—	114	239
2003	2,891,769	280,436	—	174	137
2004	2,602,554	262,192	—	101	54
2005	2,319,214	249,164	—	51	31
2006	2,240,582	251,268	—	34	8
2007	2,176,022	264,107	5,654	13	9
2008	2,163,216	315,036	5,591	12	8
2009[*1]	1,641,434	297,590	10,362	9	10
2010[*1]	1,484,778	291,002	18,322	11	5
2011[*1]	1,414,857	289,385	17,764	6	3

[*1] November of the previous year to October of the reference year.

[*2] The website of OIE, "Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide" [The website of OIE: http://www.oie.int/?id=505.].

Based on the results of surveillance in France[99,101].

Table 11. Details of BSE cattle born after the enforced feed ban was implemented

Date of birth	Year of diagnosis	Age (Months)	Surveillance stream
Jan. 2001	2006	60	Healthy slaughtered
Dec. 2001	2010	105	Fallen stock
Apr. 2004	2010	69	Fallen stock

the export of live cattle from Portugal to EU member states. In 2004, the EC lifted the ban on export from Portugal, and in 2006 from the UK, provided that certain conditions are met[113–115].

The Netherlands imposed its own regulations to ban the import of MBMs derived from ruminants originating from the UK in 1990[116,117], and in 1993, banned the presence of MBM originating from the UK, Ireland, and Switzerland in ruminant feed production plants[116].

In 1996, the export of mammal-derived MBMs from the UK to all EU member states was banned[112], and in 1998 that from Portugal was banned[113].

Due to the enforcement of the Animal By-products Regulation in 2002, the export of animal oil and fat is required to comply with a procedure to provide information on category 1 (including SRMs) and 2 (including MBMs) materials to the competent authorities of destination countries prior to exporting[118].

b) Feed control measures

The Netherlands banned the use of ruminant-derived proteins in feed for ruminants in 1989, and mammal-derived proteins in feed for ruminants in 1994. In 1999, production lines of feed for ruminants and those for non-ruminants containing animal proteins were separated from each other[116,119,120]. Since 1 July 2001, the use of animal proteins (excluding milk, dairy products, and others) and ruminant oil/fat containing more than 0.15% insoluble impurities was banned for all livestock feed[10].

However, animal oil/fat, classified as category 3 of Animal By-Products Regulation, are approved for use in livestock feed, excluding ruminant oil/fat containing more than 0.15% of insoluble impurities. In addition, preventive measures against cross-contamination are in place in slaughterhouses, rendering facilities and feed plants[10,118,121,122].

2) BSE surveillance

BSE has been designated as an official notifable disease in the Netherlands since July 1990. The Netherlands also conducts passive surveillance for BSE.

Farmers and private veterinarians must notify to the veterinary authority when they found cattle showing clinical signs of BSE including one or more definition laid in the OIE codes. In addition, clinical suspects detected at ante mortem inspections at slaughterhouses are also subjects of notification[117,123]. Consequently, 379 cattle were tested for BSE from 1991 to 2009.

In 2000, active surveillance started for risk cattle such as emergency slaughtered and fallen cattle over 24 months of age. On 1 January 2009, based on a risk assessment by EFSA, the age limit of risk cattle to be tested was raised to over 48 months[117,123]. Consequently, 622,535 of the risk cattle were tested from 2000 to 2010.

Moreover, in 2001, active surveillance was expanded to include healthy slaughter cattle aged over 30 months. Based on the risk assessment by EFSA, the age limit of healthy slaughter cattle to be tested was raised to over 48 months on 1 January 2009, and subsequently to over 72 months on 1 July 2011[117,123]. Consequently, 4,190,139 of the healthy slaughter cattle were tested from 2001 to 2010.

Sampling and diagnostic methods used in the Netherlands are in compliance with the OIE manual, EU Legislation, and the manuals of VLA.

Until 2002, all surveillance inspections were conducted at Central Veterinary Institute (CVI). Since 2003, private laboratories (currently five facilities) approved by CVI have conducted rapid screening tests for healthy slaughter cattle, and CVI has conducted confirmatory testing. Rapid screening tests on cattle categorized as others (cattle presenting clinical signs, fallen stock and others) are also conducted by CVI. CVI diagnoses using histopathological, IHC, and WB methods, which are controlled by the EU community reference laboratory (VLA) by proficiency tests. If needed, OIE confirmatory WB method is used for diagnosis[117].

Table 12 shows the annual number of cattle tested for BSE under the BSE surveillance program in the Netherlands.

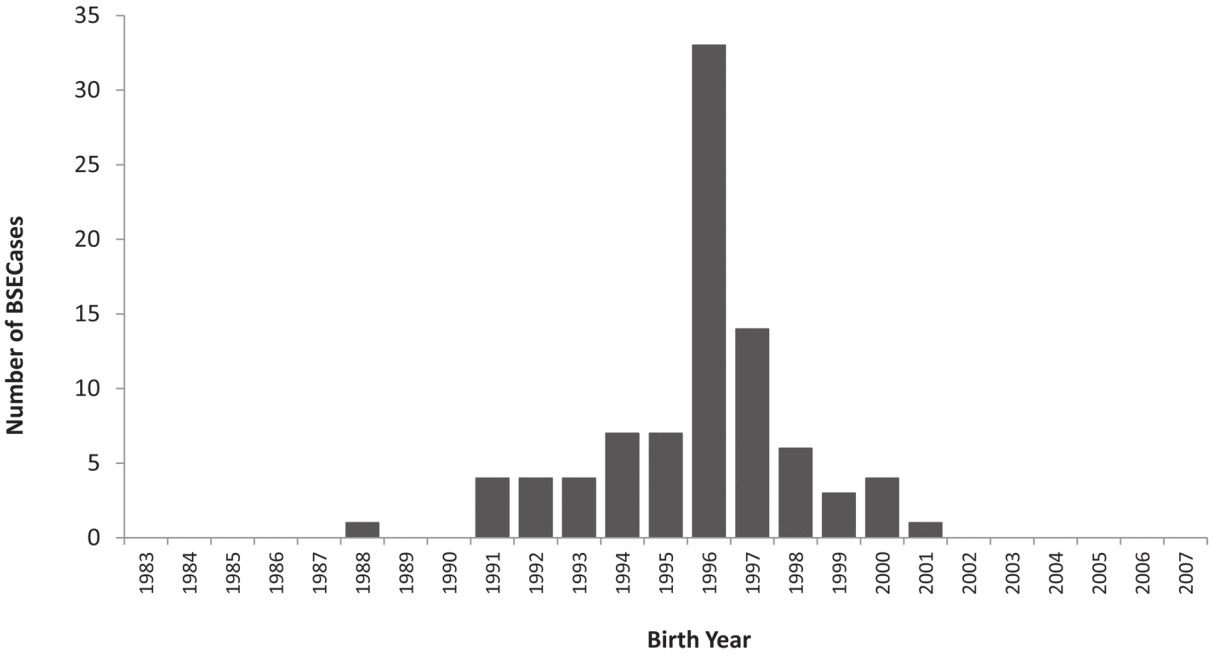


Fig. 9. Year of birth distribution of BSE cases in the Netherland

Table 12. Annual number of cattle tested for BSE under the BSE surveillance program in the Netherlands

Year	Number of cattle tested for BSE				BSE positive[*2]
	Healthy slaughter	Fallen stock	Emergency slaughter	Clinical suspect	
2001	454,649	31,056	31,281	97	20
2002	491,069	46,611	17,710	39	24
2003	441,987	49,853	15,510	25	19
2004	471,630	65,600	—[*1]	19	6
2005	455,481	47,017	17,955	7	3
2006	432,042	47,804	10,739	12	2
2007	399,304	61,413	5,230	15	2
2008	409,444	67,440	4,985	9	1
2009	357,556	44,157	3,227	4	0
2010	333,615	47,354	2,789	2	2

[*1] The number of emergency slaughter cattle in 2004 was added to that of fallen cattle.

[*2] The website of OIE, "Number of reported cases of bovine spongiform encephalopathy (BSE) in farmed cattle worldwide" **[**http://www.oie.int/?id=505**]**. Based on the surveillance program in the Netherlands[124,125].

Table 13. Details on a BSE case born after the enforced feed ban was implemented

Date of birth	Year of diagnosis	Age (Months)	Surveillance stream
Feb. 2001	2005	58	Casualty slaughter

3) BSE prevalence

a) Overview of BSE prevalence

Since the first BSE case was confirmed in the Netherlands in 1997, the number of BSE positive cattle peaked at 24 in 2002, and subsequently decreased to two in 2007, one in 2008, zero in 2009, two in 2010, and one in 2011. Until July 2012, the total number of BSE cases amounted to 88 including 19 cattle with clinical signs, 21 dead cattle and 48 healthy slaughter cattle. Among the 88 BSE positive cases, the youngest was detected at the age of 50 months and the oldest was at the age of 171 months. The average age of detection was 80 months (6.7 years). Four cattle were diagnosed with atypical BSE cases, of which one (aged 13 years) was H-type and three (aged 10, 12, 14 years) were L-type (as of November 2011)[126,127].

b) Characteristics of birth cohort

Fig. 9 shows the distribution of birth year of BSE cases in the Netherlands. **Table 13** shows the details of BSE cases detected in cattle born after feed control measures were reinforced.

A large number of BSE cases were born in 1996. Out of the cases confirmed, one was born in February 2001 after total feed ban was enforced in the same year[126]. Insufficient systems for feed production toward prevention of feed contamination and unintentional addition of pig-feed to cattle-feed in farms were suspected to be involved in infection of this case[128,129].

6. Summary of BSE Infection in Cattle Population (Table 14)

Table 14. Summary of BSE infection in cattle population (An electronic file of this table is available as a supplementary material.)

		Japan	The US	Canada	France	The Netherlands
Domestic Stability	Feeding	In April 1996, a voluntary ban on the use of ruminant MBMs in ruminant feed was imposed (administrative notice).	In 1997, the use of mammalian proteins (excluding those derived from pigs, horses, and others) for ruminants was banned.	In 1997, the use of mammalian proteins (excluding ones derived from pigs, horses, and others) for ruminants was banned.	In 1990, the use of mammal-derived proteins in feed for cattle was banned.	In 1989, the use of ruminant-derived proteins to ruminants was banned.
		In September 2001, the use of ruminant proteins in ruminant feed was prohibited (regulation).	In October 2009, the use of high risk materials such as the carcasses of BSE positive cattle, the brain and spinal cords of cattle aged 30 months or older for pet food and animal feed was banned.	In July 2007, the use of SRMs (refer to the below) for pet food, fertilizers, and feed for all livestock was banned.	In 1994, the ban was expanded to feed for ruminants.	In 1994, the use of mammalian proteins to ruminants was banned.
		In October 2001, the use of all animal-derived proteins for ruminant feed was prohibited and moreover, all the MBM import was banned (regulation).			In 1996, measures for prevention of feed contamination with SRMs, fallen cattle, and BSE cattle identified by slaughtering inspections were in place.	In 1997, the use of SRMs in all livestock feed was banned.
		Subsequently, the use of all animal proteins for ruminant feed and the use of ruminant proteins for all livestock were prohibited. .			In 2000, the use of animal proteins in all livestock feed was banned.	In 2000, the use of animal proteins in all livestock feed was banned.

Table 14. (continued.)

Use of SRMs	The head (excluding the tongue and cheek meat), spinal cord, distal ileum (two meters from the connection to caecum) of cattle of all ages, vertebral column (excluding transverse processes of thoracic and lumber vertebrae, vertebrae of the tail and the wings of sacrums) of cattle of all ages.	SRMs: The tonsils and distal ileum of cattle of all ages	SRMs: The distal ileum of cattle of all ages.The skull, brain, trigeminal ganglion, eyes, tonsils, spinal cord, and dorsal root ganglion of cattle aged 30 months or older.	SRMs: the skull (excluding the mandible but including the brain and eyes) and spinal cord of cattle aged over 12 months, vertebral column (excluding the vertebrae of tail, spinal and transverse processes of cervical, thoracic, and lumbar vertebrae, the median sacral crest, and wings of sacrum but including the dorsal root ganglia) of cattle over 30 months of age.	SRMs: the skull (excluding the mandible but including the brain and eyes) and spinal cord of cattle aged over 12 months, vertebral column (excluding the vertebrae, of tail, spinal and transverse process of cervical, thoracic, and lumbar vertebrae, the median sacral crest, and wings of sacrum but including the dorsal root ganglia) of cattle over 30 months of age. The tonsils and intestines from the duodenum to the rectum and mesentery of cattle of all ages.
	In October 2001, the removal and incineration of the head (excluding the tongue and cheek meat), spinal cord, and distal ileum (two meters from the connection to the caecum) of cattle of all ages became compulsory.	The brain, skull, eyes, trigeminal ganglia, spinal cord, vertebral column (excluding tail vertebrae, thoracic and lumbar transverse processes, and sacral wings), and dorsal root ganglion of cattle aged 30 months or older.	Until 2007, SRMs of healthy slaughter cattle aged less than 30 months, and that of fallen cattle removed at slaughterhouses were used in feed for pigs and chickens after rendering.	The tonsils and intestines from duodenum to the rectum and mesentery of cattle of all ages.	All the SRMs are removed and incinerated after being rendered at rendering facilities.
	In January 2004, the removal of vertebral column became compulsory. SRMs were required to be incinerated at 800 °C or more.	Until 2009, SRMs were used in feed for pigs and chickens after rendering.	In July 2007, the use of SRMs for pet foods, fertilizers, and all animal feed was banned.	From 1996, all the SRMs are incinerated after being processed at dedicated rendering facilities, and prevented from being mixed in food and feed. SRMs other than the vertebral column are removed with dedicated tools at slaughterhouses and disposed in dedicated containers. The vertebral column is removed at meat processing facilities.	

Table 14. (continued.)

		In October 2009, the use of carcasses of BSE positive cattle and high risk materials such as the brain and spinal cord for pet food and animal feed was banned.			
Rendering Conditions	The use of ruminant MBMs in all livestock feed is banned and rendering processes of ruminants are segregated from those of pigs and chickens.	Since 1997, it became compulsory to keep equipment separately, or to wash production lines.	A continuous rendering system (104–146 °C, 0.5–1 bar, 20–180 min.) and a batch system (156/275 °C, slightly vacuumed, 120/165 min.) are installed.	In 1991, it became compulsory to incinerate high risk materials.	Until 1989, a batch system was used at all rendering facilities.
	The MBMs are used in cement production at the cement factory or incinerated at waste disposal plants.		CFIA inspectors are present at facilities where prohibited or non-prohibited materials other than SRMs are produced.	From 1993, high risk materials must be rendered at 133 °C, 3 bar, 20 min. after grinding in the size smaller than 50 mm.	In 1989, a continuous rendering system was replaced with the batch system at some rendering facilities.
				From 1996, in accordance with the European Commission Decision (1996/449/EC), it is compulsory to render all waste materials from cattle at 133 °C, 3 bar, 20 min. after grinding in the size smaller than 50 mm. Carcasses of all animals died in farms and those not slaughtered for human consumption, and SRMs were designated as high risk materials, and the incineration became compulsory.	In 1995, high process standards for processing ruminant waste materials were adopted.

Table 14. (continued.)

				From 1998, all mammalian animal waste to be used for producing MBMs in feed were obliged to be rendered at 133 °C, 3 bar, 20 min after being ground in the size smaller than 50 mm.	In 1996, a batch system (133 °C, 3 bars, 20 min) was installed in all the rendering facilities.
					In December 1997, it became compulsory to implement an autoclaved sterilization (133 °C, 3 bars, 20 min.) for mammalian animal waste that are used for producing MBMs. From August 1997, all animal by-products were obliged to be rendered at 133 °C, 3 bars, 20 min. after being ground in the size smaller than 50 mm.
Cross-contamination control measures	In June 2003, a law was promulgated to separate production lines for ruminant feeds from those for other livestock feeds. Provisional measures were in place until 2005.	In 2005, 80% of the rendering facilities (205/255) and 99% of the feed plants (6,121/6,199) were dedicated to processing either prohibited or non-prohibited materials.	In 1997, ruminant feed plants as well as plants producing non-ruminant feed including prohibited materials were separated and production lines were also separated. It became compulsory to clean production lines. Feed manufacturers and the owners of ruminants must keep records on prohibited materials such as the date received and maintain records of purchase amounts and purchase dates of all feed and feed materials.	At facilities producing non-ruminant feed using fish meal, di- and tri-calcium phosphates, and blood products, all of which are prohibited to use in ruminant feed, the facilities and production lines must be separated to prevent cross-contamination to ruminant feed.	In 1993, production of ruminant feed using the same mixers as those used in producing feed containing more than 6% MBM was prohibited.

Table 14. (continued.)

	In April 2005, the separation of the feed production lines was completed with implementation of separation of the processing of pigs.	In 2009, more facilities and plants were dedicated to processing either prohibited or non-prohibited materials. Consequently less than 2% of them use cleaning as a method for prevention of cross-contamination.	In 2005, the number of dedicated plants increased, and 79% of rendering facilities (23/29) and 83% of feed plants (456/550) were dedicated to processing prohibited or non-prohibited materials.	In 2008, 20 facilities were producing feed containing animal-derived materials. Out of 20, 18 facilities were producing non-ruminant feed and 2 were producing feed for both non-ruminant and ruminants. Production lines in these 2 facilities are physically separated to prevent cross-contamination	At facilities producing ruminant feed, the use of MBMs imported from the UK, Ireland, and Switzerlandwas was banned.
			In 2007, it became compulsory to separate ruminant feed plants as well as plants producing non-ruminant feed including prohibited materials, and also to separate production lines.		In 1999, production lines for ruminant feed were fully separated from those for non-ruminant feed.
					By 2011, four facilities were producing non-ruminant feed including animal proteins approved for use. All of the four facilities have separated production lines.
Surveillance Outline	BSE testing is conducted on all cattle of all ages slaughtered at slaughterhouses and all fallen cattle aged 24 months or older.	High risk cattle such as those exhibiting central nervous system signs and fallen cattle are tested.	High risk cattle such as those exhibiting clinical signs, fallen cattle, and emergency slaughter cattle are tested.	Cattle showing clinicalsigns, fallen cattle, and emergency slaughter cattle, all of which are aged over 24 months, are tested.	Cattle showing clinical signs, fallen stock on farm, and emergency slaughter cattle, all of which are aged over 48 months (over 24 months until December 2008), are tested.
	In FY 2011, the number of cattle tested at slaughterhouses was approx. 1.19 million (accumulated total: approx. 12.57 milion).	USDA implements a surveillance program designed to detect BSE at a prevalence level of one case per one million cattle.	The surveillance program is designed to detect at least one BSE positive cattle at a confidence level of 95% where the prevalence of BSE is two per one million adult cattle.	In July 2011, the age limit for testing on healthy slaughter cattle was raised from 48 to 72 months.	In July 2011, the age limit for testing on healthy slaughter cattle was raised from 48 to 72 months.

Table 14. (continued.)

	In FY 2011, the number of dead cattle and others which were tested was approx. 0.1 million (accumulated total: Approx. 0.83 million)	The number of tested cattle: 40,000 mil./ year	After 2005, 30,000 or more cattle have been tested annually.	The surveillance program is sufficient to meet a level of the OIE point system for detection of BSE at least one in 0.1 million adult cattle.	The surveillance program is sufficient to meet a level of the OIE point system for detection of BSE at least one in 0.1 million adult cattle.
	The surveillance program is sufficient to meet a level of the OIE point system for detection of BSE at least one in 0.1 million adult cattle.	The surveillance program is sufficient to meet a level of the OIE point system for detection of BSE at least one in 0.1 million adult cattle.	The surveillance program is sufficient to meet a level of the OIE point system for detection of BSE at least one in 0.1 million adult cattle.		

V. SRM and Meat Processing Process

1. Japan

1) Removal of SRM

a) Methods for removal of SRM

In Japan, it is compulsory to remove the head (excluding the tongue and cheek meat), spinal cord, and distal ileum [two meters length from the ileocecal junction] as SRMs from cattle of all ages according to the "Abattoir Law Enforcement Regulation (Ordinance of the MHLW, No. 44, Ministry of Health, Labour and Welfare, 1953)" and the "Enforcement Regulation for the Law on Special Measures Against Bovine Spongiform Encephalopathy under the Jurisdiction of the Ministry of Health, Labour and Welfare (Ordinance of the MHLW, No. 89, Ministry of Health, Labour and Welfare, 2002)". It is also compulsory to remove the vertebral column (excluding the transverse processes of thoracic and lumbar vertebrae, vertebrae of tail, and the wings of sacra) of cattle raised in BSE-occurring countries and regions, when their meat is sold to consumers[6,130–132].

Furthermore, it is required according to the "Enforcement Regulation for the Abattoir Law" to remove SRM to prevent the contamination of edible parts during slaughter, to keep them in a dedicated container, and to incinerate them completely at over 800 °C after inspection by inspectors (veterinarian of the local government)[130,131].

Generally, the spinal cords are removed by suction from carcasses prior to carcass splitting. After carcass splitting, carcasses are washed with high-pressured water and checked to insure that no remnants of spinal cords are present. Carcass splitting saws are washed after each splitting operation[131,133].

b) Control based on sanitary standard operation procedure (SSOP) and hazard analysis and critical control point (HACCP)

All slaughterhouses must keep the SSOP to minimize BSE risks derived from SRM. Inspections are therefore conducted at intervals specified by the SSOP, and their records are maintained[133].

2) Slaughtering process

a) Inspection before slaughter and BSE testing at slaughterhouses

Ante-mortem and post-mortem inspections of slaughtered animals are conducted at slaughterhouses.

In the ante-mortem inspection, all cattle are inspected for strange cries, behavioral abnormalities such as circling, clinical signs of CNS impairment including ataxia, and gait patterns. When BSE is clinically suspected, the suspected cattle must not be slaughtered in compliance with the Ordinance the Abattoir Law (No. 114, 1953)[45,134].

BSE inspection is conducted on healthy slaughtered cattle of all ages (voluntarily conducted on cattle aged 20 months or younger) during postmortem inspection of them. Furthermore, meat and offal (including separated parts to be disposed) derived from cattle must be kept during inspection in a way able to identify individual animals and to prevent microbial contamination of edible parts[45].

b) Stunning and pithing

Among 149 slaughtering facilities, 141 facilities use stun-guns (slaughtering guns) and 15 use hammers, while a device injecting compressed-air or gas into the cranial cavity is not used in any slaughterhouses. Out of 141 slaughterhouses using stun guns, 140 use guns which insert the tip of the bolt into the cranial cavity, and three [The numbers are overlapping due to facilities that use plural methods] use guns which do not insert the tip ("Results of the survey on the handling of specific materials" as of March 2012)[133].

Since 1 April 2009, pithing (destruction of brain and spinal cord using wires or equivalent equipment) has been prohibited by item (iii), paragraph 1, Article 7 of the Ordinance for Enforcement of the Abattoir Law[135].

According to the "Result of on-the-spot inspection regarding pithing (June 2009)" (MHLW), pithing is not used in any slaughterhouse as of March 2009[136].

3) Others

a) Mechanically recovered meat (MRM)

MRM is not produced in Japan[137].

The method for the removal of the vertebrae column is specified in the Standards and Criteria for Food and Food Additives (notification of the Ministry of Health, Labor and Welfare No. 370, 1959). The method must be able to prevent contamination of the meat, edible offal, and meat around the vertebrae column, with the dorsal root ganglia tissues[132,138].

b) Traceability

The age of cattle is determined during inspection at slaughterhouses according to the "Summary of inspection methods of transmissible spongiform encephalopathy" by examining the dentition and referring to the copy of individual cattle identification register records. Cattle are judged as 30 months or older if they have a third incisor, regardless of the age description in the individual register records.

The Japanese traceability system started in January 2002. The management system of individual identification of cattle by using a registration system (individual cattle identification register) was constructed in accordance with the Law for Special Measures Concerning the Management and Relay of Information for Individual Identification of Cattle (Law No. 72, 2003), and became mandatory in production stages in December 2003 and also in distribution stages in December 2004[16,45].

c) Number of slaughterhouses and head-count of slaughtered cattle

There are 149 slaughterhouses in Japan as of March 2012. Approximately 1.22 million heads of cattle are slaughtered annually, of which 0.86 million are aged 30 months or younger (as of 31 May 2011)[47,133].

2. The US

1) Removal of SRM

a) Methods for removal of SRM

In the US it is compulsory to remove the brain, skull, eyes, trigeminal ganglion, spinal cord and vertebral column (excluding tail vertebrae, thoracic and lumber transverse processes, and sacral wings), and dorsal root ganglia of cattle aged 30 months or older, and the tonsils and distal ileum of cattle of all ages.

Carcasses are split during the slaughtering process. The spinal cord must be removed by suction after carcass splitting, and then carcasses are washed with warm or cold water. The carcass splitting saw must be washed after splitting each carcass. Employees and inspectors visually check to insure that no portions of spinal cord tissues are remaining in the vertebral column, and inspectors, including veterinarians, visually inspect to confirm the removal of SRM[17].

b) Control based on SSOP and HACCP

Slaughterhouses must incorporate HACCP or SSOP plans, and maintain a system including the implementation of monitoring and recordkeeping according to Federal Regulations 9CFR310.22[139].

HACCP programs have been established in facilities certified to export beef to Japan, and on-site inspection of these facilities has been conducted to confirm the implementation status[40].

c) Additional requirements etc for export to Japan

Japan banned the import of beef from the US in 2003 after a BSE case was found in the US. In 2005, based on the risk assessment of the FSCJ, Japan resumed the import on certain conditions (the removal of SRM from cattle of all ages and beef certificated to be derived from cattle aged 20 months or younger).

To meet the requirements for export to Japan, the United States Department of Agriculture (USDA) developed an Export Verification (EV) Program. Only facilities that meet the requirement specified by the EV program may export products to Japan. Main requirements are removal of SRM [SRM defined by Japan (head of cattle of all ages (excluding the tongue and cheek meat), spinal cord, distal ileum (two meters from ileal-cecal junction), and vertebral column.) Hereinafter same for Canada.] from cattle of all ages, and a certificate including a statement certifying that products are derived from cattle aged 20 months or younger at the time of slaughter. The age of cattle needs to be verified by live animal production record[141].

2) Slaughtering process

a) Inspection before slaughter and BSE inspection at slaughterhouses

All cattle brought to slaughterhouses are visually inspected for gait patterns by veterinarians or meat inspectors under the supervision of veterinarian. Cattle exhibiting clinical signs of CNS impairment, fallen cattle, and downers are prohibited to be slaughtered for human consumption[17].

BSE testing on all healthy slaughter cattle aged 30 months or older was conducted until 2006, but not since 2007[17,65,142].

b) Stunning and pithing

Penetrating captive bolt stun-guns are used in most slaughterhouses. Compressed-air stun-guns with air injection have been prohibited in the US since January 2004, because it may force visible pieces of brain into the circulatory system of stunned cattle. Human Slaughter Act in the US[17] has prohibited the use of pithing.

3) Others

a) MRM

MRM produced in the US is not exported to Japan. The skull and vertebral column of cattle aged 30 months or older **[**Federal Regulations 9CFR 319.5, http://ecfr.gpoaccess.gov/cgi/t/text/textidx?c=ecfr&sid=0104c05e673aafd200080564340b0a26&rgn=div8&view=text&node=9:2.0.2.1.20.1.22.3&idno=9**]** is prohibited from the use in MRM production in the US.

b) Traceability

In the US, cattle ages are determined by confirmation of documents and examination of dentitions. As written in FSIS Notice 5–04, cattle with eruption of at least one of the second set of permanent incisors (I2) is judged as those at 30 months of age or older. Although inspection personnel are not required to check dentition on each animal, they should periodically verify that dentitions are examined in a correct and accurate manner in facilities by reviewing records, observing employees to perform dentition examinations, and periodically conducting dentition checks[139].

In the US, for many years, individual livestock identification has been conducted as a monitoring scheme for animal diseases such as cattle tuberculosis and brucellosis. In April 2006, the USDA announced to launch a National Animal Identification System (NAIS). The system is designed to identify infected livestocks and farms rearing them within 48 hours of the occurrence of animal diseases such as a foot-and-mouth disease. NAIS was operated by the USDA to aim to construct a uniform system in the US, but the system was based on voluntary participation so that the participation rate in domestic animal producers remains low at 36% as of February 2010. Subsequently, in February 2010 the USDA introduced a new animal disease traceability system to be operated by each state. This system requires mandatory participation. From 11 August to 9 December 2011, the USDA called for public comments for its legislation[143].

c) Number of slaughterhouses and slaughtered cattle

As of June 2012, 58 facilities are eligible to export beef to Japan. During 2005 and 2011, surveys and inspections were carried out on 155 facilities to confirm and verify the compliance with requirements for beef export to Japan (the status of age determination and removal of SRMs)[140,144].

According to data of 2011, approximately 34.94 million cattle were annually slaughtered, approximately 34.09 million of which were adult cattle over one year of age[145].

3. Canada

1) Removal of SRM

a) Methods for removal of SRM

In Canada, the distal ileum of cattle of all ages, the brain, skull, eyes, tonsils, trigeminal ganglia, spinal cord, and dorsal root ganglia of cattle aged 30 months or older are defined as SRMs[86,146]. The spinal cord is removed by suction after carcass splitting. After removal of spinal cord, carcasses are generally washed with warm or cold water, and visually checked by employees or inspectors to ensure that no portions of spinal cord tissues are remaining in the vertebral column. The carcass splitting saw must be washed after splitting each carcass. Subsequently, inspectors including veterinary officers visually inspect for the removal of other SRMs from the carcasses[17,147].

The SRMs are stained immediately after removed, and placed in a dedicated container clearly marked with the word "SRM". The methods approved for SRM destruction are burial, incineration, alkaline hydrolysis and thermal hydrolysis[86,146].

b) Control based on SSOP and HACCP

Since November 2005, registered establishments are required in compliance with the Meat Inspection Regulations (MIR) to establish, develop and maintain HACCP. It is prescribed that the HACCP systems should meet the requirement of the Food Safety Enhancement Program (FSEP) of CFIA[146].

c) Additional requirements etc for export to Japan

Japan banned the import of beef from Canada in 2003 after a BSE-positive cow was found in Canada. In 2005, based on the risk assessment of the FSCJ, Japan resumed the import on certain conditions (the removal of SRM derived from cattle of all ages and certificates including statement that beef is derived from cattle aged 20 months or younger).

"Standards for the Slaughter of Cattle and Processing of Beef Products Eligible for Export to Japan (CFIA, 16 May 2005)" was developed to meet the requirements for export to Japan. Only companies that meet the specified product requirement under the standards may export products to Japan. Main requirements are the removal of SRMs from cattle of all ages and certification. Certificates should include statement that products are derived from cattle aged 20 months or younger at the time of slaughter. The age of cattle must be verified by live animal production records.

2) Slaughtering process

a) Inspection before slaughter and BSE testing at slaughterhouses

All cattle brought to slaughterhouses are visually inspected for gait patterns by veterinarians or meat inspectors under their supervision. Cattle exhibiting clinical signs of CNS impairment, fallen cattle, or downers are prohibited to be slaughtered for human consumption[17,148]. Since the BSE surveillance program started in Canada, healthy slaughter cattle have not been tested for BSE[86].

b) Stunning and Pithing

Penetrating captive bolt stun guns are used in most slaughterhouses. Compressed-air stun guns with air injection have been prohibited since 2000 because it may cause the entrance of pieces of brain tissues into the circulatory system of stunned cattle.

Pithing is prohibited by the Meat Inspection Regulations[86,148].

3) Others

a) Mechanically recovered meat (MRM)

MRM produced in Canada is not exported to Japan.

The skull and vertebral column of cattle aged 30 month or older are prohibited from the use in production of MRM in Canada[17].

b) Traceability

In Canada, cattle are considered as younger than 30 month of age as long as the third permanent incisor is not above the surface of gum line[146].

In 2001, the Canadian Cattle Identification Program (CCIP), a livestock trace back system, was introduced throughout Canada except in Quebec. It was designed to detect, prevent, and eradicate livestock diseases.

Since 2002, the program has been fully implemented, and under a regulatory requirement, all cattle have been identified with ear tags. A violation of the requirement may result in fines. Currently, the rate of compliance with the identification program is 97% or over.

Quebec has its own identification system. The Agri-Traçabilité Québec (ATQ) manages a database (ATQ database) containing information on various animals. All cattle must be identified by two ear tags (a dangle tag and radio frequency identification tag), tagged at the farm where the animal was born or upon arrival to its destination in Quebec. The ear tags used in Québec are approved by the Canadian Cattle Identification Agency and are also used in other states[147,149–152].

c) Number of slaughterhouses and slaughtered cattle

A total of 35 slaughter establishments are federally registered. CFIA staffs verify the operator's compliance with the regulations through the completion of relevant inspection tasks and audits[146]. Out of the 35 facilities, 12 are eligible

to export beef to Japan (as of November 2011)[153]. According to the data of 2009, the annual number of cattle slaughtered was approximately 3.41 million. Approximately 3.14 million of them including 0.6 million of cattle over 30 months of age were slaughtered in federally registered slaughter establishments[146].

4. France

1) Removal of SRM

a) Methods for removal of SRM

In France, SRMs are defined as follows: the skull (excluding the mandible but including the brain and eyes) and spinal cord of cattle aged 12 months or older; the vertebral column (excluding the vertebrae of tail, spinous and transverse processes of cervical, thoracic, and lumbar vertebrae, the medium sacral crest, and wings of sacrum, but including the dorsal root ganglion) of cattle aged over 30 months; and tonsils, intestines from duodenum to rectum, and mesentery of cattle of all ages[104].

The SRM removal in accordance with the SRM Guide for cattle in slaughterhouses are inspected and supervised by inspectors of DDVS. Methods of SRM removal are verified by DGAL and AFSSA **[**On 1 July 2010, AFSSA and gence Francaise de Securite Sanitaire de l'Environnement et du Travail (AFSSET) were merged into Administracion Nacional de la Seguridad Social (ANSES).**]**.

It is compulsory to carry out suction removal of spinal cord prior to carcass splitting for cattle aged over 12 months. When any spinal cord remnant is discovered after carcass splitting, operators must remove the remnants, and at carcass inspection, veterinary officer must confirm the complete removal of all spinal cord. After the removal of the spinal cord, carcasses are washed with a steam vacuum, but not with high-pressured water. Carcass splitting saws must be washed after splitting each carcass. The meat inspector confirms whether the SRMs other than the vertebral column were successfully removed from the carcasses at slaughterhouses. Removed SRMs are disposed in a dedicated container. The vertebral column of cattle aged over 30 months is removed at meat processing facilities[154–156].

b) Control based on SSOP and HACCP

Good Hygiene Practice (GHP) **[**GHP is similar to SSOP with different form of expressions.**]** must be implemented at all slaughterhouses and meat processing plants.

HACCP has been incorporated in all slaughterhouses since 2001 and in meat processing facilities selling products directly to consumers since 2006[154].

The local branches of competent authorities evaluate the conformity of HACCP plan with the enforced regulation[155].

c) Additional requirements etc for export to Japan

Japan has suspended the import of beef from the EU countries since 2000. Accordingly, no beef has been imported from France to Japan as of 2012.

2) Slaughtering process

a) Inspection before slaughter and BSE inspection at slaughterhouses

All cattle brought to slaughterhouses are visually inspected for gait patterns by veterinarians of DDVS. BSE suspect cattle displaying clinical signs such as apprehension, fear, fright, hyperesthesia, and ataxia are segregated, and euthanized, and subsequently samples are taken from suspected cattle for BSE tests[155].

BSE surveillance has been implemented including BSE testing on healthy slaughter cattle aged over 30 months from January 2001, those aged over 24 months from July 2001, those aged over 30 months from August 2004, those aged over 48 months from January 2009, and those aged over 72 months from July 2011[101].

b) Stunning and pithing

The EU legislations and the French laws ban the use of stun guns for stunning animals intended for human consumption, and therefore no slaughterhouses use stun-guns or livestock stunners. All facilities use either captive bolt pistols (bolt enters the skull) or concussion pistols (bolt does not enter the skull), and a stunning hole is sealed with a durable and waterproof stopper[154,155].

Pithing is banned by the EU legislations and the French laws[154,155].

3) Others

a) MRM

Based on the EU legislation, the use of ruminant bones from countries with a controlled or undetermined risk is prohibited for the production of mechanically recovered meat (referred to as MSM under the EU legislation)[95,154].

b) Traceability

In France, ear tags and personal cattle passport information are used to identify the age of cattle. Dentition is not used as an official mean for age identification[155]. Ear tags with individual identification numbers have been given to all cattle aged 6 months or older since 1969. In 1995, all cattle-raising farms were registered, and two ear tags were to be attached to each cow, one within 48 hours from the birth and the other within four months from the birth. In 1998, every place, where cattle are kept, was registered, and two ear tags were to be attached within seven days of birth, and all cattle were required to be accompanied by their passports specifying their mothers' individual identification numbers. In 2006, the regulation was revised and farmers were allowed to attach the ear tags within 20 days of birth to harmonize with the EU legislations[9].

c) Number of slaughterhouses and slaughtered cattle

DDVS grants final approval for registration of slaughterhouses and meat processing plants in accordance with national standards based on Regulation (EC) No854/2004. In France as of 2009, 259 slaughterhouses and 1,208 meat processing plants are operated in accordance with the EU legislation[155].

According to the data of 2006, approximately 5.13 million cattle are annually slaughtered, approximately 2.33 million of which are aged over 30 months[154].

5. The Netherlands

1) Removal of SRM

a) Methods for removal of SRM

The skull (excluding the mandible, but including the brain and eyes) and spinal cord of cattle aged over 12 months, the vertebral column (excluding the vertebra of tail, spinous and transverse processes of cervical, thoracic and lumbar vertebrae, the median sacral crest, and the wings of sacrum, but including the dorsal root ganglion) of cattle over 30 months of age, and the tonsils, the intestines from the duodenum to rectum and mesentery of cattle of all ages are specified as SRM.

After the splitting of carcasses, the spinal cord is manually removed from the spinal canal using a small metal scraper. Then the spinal canal is cleaned with a vacuum cleaning device. The removal of the spinal cord is confirmed by inspectors. Carcass splitting saws are washed for each animal, but carcasses are not washed with water after the removal of the spinal cord.

The tonsils, the intestines including distal ileum, and mesentery are removed by trained employees and confirmed by inspectors.

The removal of SRM is inspected and supervised by the inspectors of Nederlandse Voedsel- en Warenautoriteit (VWA). Removed SRMs are rendered and then incinerated[157–160].

b) Control based on SSOP and HACCP

In the Netherlands, all facilities must introduce HACCP. Large facilities develop their own HACCP plans, while many small facilities do it in accordance with the Dutch Hygiene Code made by the Product Boards for Livestock (a trade organization in the Netherlands). The HACCP plan of each facility is certified by VWA, and reassessment and modification of the HACCP plan are also audited by VWA. The frequency of the reassessment and modification varies by facility[157,160].

c) Additional requirements etc for export to Japan

Japan has suspended the import of beef from EU countries. Accordingly, no beef has been exported from the Netherlands to Japan as of 2012.

2) Slaughtering process

a) Inspection before slaughter and BSE inspection at slaughterhouses

All cattle brought to slaughterhouses are visually inspected for gait patterns by the veterinarians of VWA. BSE suspect cattle displaying clinical signs such as fear, apprehension, hyperesthesia, and ataxia are not slaughtered at slaughterhouses, but are transported to the Central Veterinary Institute (CVI) to be euthanized and tested for BSE[160].

BSE testing on healthy slaughter cattle over 30 months of age started in January 2001, those over 48 months of age from January 2009, and those over 72 months of age from July 2011[123,159].

b) Stunning and Pithing

All facilities use stun guns shooting metal pins, but not those shooting compressed air inside the skull. Pithing is not allowed in the Netherlands[158,160].

3) Others

a) MRM

According to the EU legislation, production of mechanically recovered meat from cattle (including calves) is prohibited[157,161].

b) Traceability

In the Netherlands, dentition is not used to identify the age of cattle, but the Identification and Registration (IR) system is used. The IR system introduced in 1990 is a national central database including records of registration and movement of all cattle. In 2000, the system was changed in accordance with the Regulation (EC) No 1760/2000 of the European Parliament and the Council, and since then all domestic and imported cattle are traceable (back to their farms of origin). All farms must register calves within three days of their birth[117,159].

c) Number of slaughterhouses and slaughtered cattle

As of 2010, nine facilities annually slaughter 10,000 or more adult cattle, four facilities 0.1 million or more calves aged 8 months or younger, and three facilities 25,000 or more calves aged from 8 to 12 months[123].

According to the data of 2010, approximately 2.03 million cattle are annually slaughtered. Among them, 0.54 million are adult cattle over 12 months of age, approximately 1.24 million are younger than 8 months of age, and 0.25 million are from 9 to 12 months of age[123].

5. Summary (Table 14)

VI. Atypical BSE

1. Background

Recently, a few cases of BSE (atypical BSE) demonstrating distinct PrP^{Sc} band patterns on WB from conventional BSE have been reported in Europe, Japan, the US and others. Atypical BSE is classified into two types, H-BSE and L-BSE (BASE), based on their molecular weight [Molecules of classical PrP^{Sc} have two sites for glycosylation, and three band patterns (unglycosylated, monoglycosylated, and diglycosylated PrP^{Sc}) are detected by WB] of unglycosylated prion proteins (PrP^{Sc}) [Molecular weight of unglycosylated PrP^{Sc} is 20 kDa in classical BSE, 21 kDa in H-BSE showing a WB band at a higher position than that of classical BSE, and 19 kDa in L-BSE showing a WB band at a lower position than that of classical BSE.][162–164].

Conventional BSE is defined as “classical BSE” to clearly distinguish it from atypical BSE.

In Japan, two cases of L-BSE have been reported. One is a Holstein steer aged 23 months (BSE/JP8) and the other is a Japanese black cow aged 169 months (BSE/JP24). No H-BSE cases have been reported (as of July 2012). BSE/JP24 showed dysstasia and was diagnosed to be positive by the BSE rapid test at a slaughterhouse. As described in the “III. Scientific findings from laboratory studies”, no clinical signs were observed in BSE/JP8 and this case was diagnosed with a pseudopositive case based on the result of BSE rapid tests at a slaughterhouse. The accumulation of PrP^{Sc} in the BSE/JP8 brain was only a little so that the remaining brain portions after ELISA test were analyzed by WB following

the precipitation with phosphotungstic acid, and consequently this case was concluded to be atypical BSE. The accumulation of PrP^{Sc} in the obex of BSE/JP8 was scarce and estimated to be approximately 1/1,000 of that of BSE/JP6. No infectivity was observed by bioassays using TgBovPrP mice over-expressing bovine PrP[3,4,165].

The findings of atypical BSE in risk assessments conducted hitherto by FSCJ based on information collected up to 2010 is summarized as follows[164].

- Most cases of atypical BSE have been detected in animals over eight years old, ranging from 6.3 to 18 years with an exception of the cow aged 23 months found in Japan.
- Frequencies of H-BSE and L-BSE in France were 0.41 and 0.35, respectively, per one million adult cattle tested. Among cattle found at their age over 8 years, the frequencies of H-BSE and L-BSE were 1.9 and 1.7, respectively, per one million.
- L-BSE and H-BSE prions are able to be transmitted when intracerebrally inoculated to mice, Tg mice over-expressing bovine or ovine PrP, or inbred mice. L-BSE prions have been shown to transmit easily to Tg mice over-expressing human PrP and primates, and indicated to be possibly more pathogenic than classical BSE.

The Committee considered several new studies that had become available since the previous assessments of BSE. These included the property, distribution, infectivity, and epidemiological characteristics of atypical BSE prions.

2. Properties of Atypical BSE Prions and Distribution of the Protein in Infected Cattle

Histological distribution of PrP^{Sc} in cattle challenged with L-BSE agents was examined by i.c. inoculation with 1 mL of 10% homogenate of the medulla oblongata from a L-BSE case identified in Japan (BSE/JP24) to five calves (Holstein aged 2 to 3 months). The challenged animals were slaughtered at 10, 12, and 16 mpi to collect their tissues. Tissue PrP^{Sc} was precipitated with phosphotungstic acid and analyzed by WB. Accumulation of PrP^{Sc} was observed in the CNS and peripheral nerve tissues at all the time points, but not in the lymphatic tissues including the spleen[166].

Histological distribution of PrP^{Sc} in cattle infected with L-BSE and H-BSE has been studied by i.c. inoculation with 1 mL of 10% homogenate of the brainstem from L-BSE and H-BSE cases identified in Germany to six Holstein/Friesian cattle. All challenged cattle presented clinical signs at 14 mpi. Accumulation of PrP^{Sc} was detected by WB in the brainstem of all the animals except one slaughtered at five mpi. Analysis by ELISA test demonstrated that there was no accumulation of PrP^{Sc} in the peripheral nerve, tonsils, spleen, ileum Peyer's patch, tongue nerve tissue, and musculus semitendinosus[167,168].

Distribution of PrP^{Sc} in the brain of BASE (L-BSE) prion identified in Italy has been studied by i.c. inoculation with 1 mL 10% homogenate of the brain (thalamus) from a BASE case aged 15 years [BASE: BSE cattle found in Italy with biochemically and pathologically different characteristics from classical BSE. They were named bovine amyloidotic spongiform encephalopathy (BASE) from the characteristics of amyloid vacuolar degeneration and kuru plaques onrostrum structure including thalamus and olfactory bulb found as a result of IHC.] to six calves of Friesian and Alpine Brown aged 4 months. For comparison, brain homogenate of a cow affected with classical BSE was intracerebrally inoculated to six calves. In the calves challenged with the BASE-affected brain homogenate, neurological signs were observed at 461 to 551 days post-inoculation. Regarding i.c. accumulation of PrP^{Sc}, only a small amount of PrP^{Sc} was found in the cerebrum and cerebellum of calves challenged with the classical BSE agent, but a large amount was found in the cerebrum, cerebellum, and hippocampus of calves challenged with BASE agent[169].

The presence of infectivity in BASE-affected muscles was studied by bioassay using TgbovXV Tg mice. The bioassay was conducted by i.c. (20 μL) and i.p. (100 μL) inoculation of 10% homogenate of muscles from two asymptomatic BASE cows of 14 and 13 years old identified by active surveillance in Italy, and also from one of the above mentioned experimentally challenged cattle. Brain homogenate from the three BASE cattle was also inoculated as a positive control into 5 TgbovXV mice. The brain homogenates caused infection in all the five challenged mice with the average survival time 211 to 215 days. As for the mice inoculated with the muscles from the natural BASE case aged 14 years, one out of seven mice inoculated with the *gluteus* muscle, and one out of nine mice inoculated with the *intercostalis* muscle developed clinical signs of disease. The survival time of the 2 mice was 396 days and 541 days. Additionally, five out of seven mice inoculated with *longissimus dorsi* muscle of the experimentally-infected BASE cow developed clinical signs of disease with survival time of 410 days, but no infectivity was observed in mice inoculated with the cervical lymph nodes. Infectivity was not detected in the spleen and kidney of any cattle examined. In the experimentally-infected BASE cow, PrP^{Sc} accumulation was detected by IHC in the *longissimus dorsi* and *pectoralis profundus* muscles, but not in any muscles of fore and hind limbs. On the other hand, PrP^{Sc} accumulation was detected by IHC in the *trapezius*, *biceps femoris*, *semitendinosus*, and *peroneus* muscles of the natural BASE case of 13 years old, but not in 11 muscles of

forelimbs, thoracic, buttocks, abdomen, and hindlimbs. The accumulation of PrP^{Sc} was found in the cytoplasm of muscle fibers of the peroneal muscle[170].

In order to clarify the distribution of prions in H-BSE cattle, the brain homogenate of a H-BSE cow identified in Canada was intracerebrally inoculated to 3 calves of Holstein aged 3 to 4 months. Clinical signs of early stage were observed at 12 mpi in these experimentally-infected cattle. The challenged animals were slaughtered at 507 to 574 days post-inoculation, and the tissues were collected for examination of PrP^{Sc} accumulation. Among these tissues, accumulation of PrP^{Sc} was found in the CNS, spinal cord nerve, cauda equina, DRG, and peripheral nerve tissues such as trigeminal ganglion, but not in the lymphatic tissues[171].

A type of BSE which was different from either classical BSE or conventional atypical BSE (H-BSE and L-BSE) [WB analysis showed that the molecular weight of unglycosylated PrP^{Sc} is 16 kDa, monoglycosylated PrP^{Sc} is 20 kDa, and diglycosylated PrP^{Sc} is 25 kDa.] has been reported based on WB patterns of the brainstem of two BSE cases[172]. One was an asymptomatic cow that died at a farm in Switzerland at the age of eight years in 2011 and determined to be BSE positive by postmortem examination. Another was a cow aged 15 years old and confirmed as BSE positive at a slaughterhouse. However, the details of these cases have not been reported.

3. Infectivity of Atypical BSE Prions

1) Experimental infection in mice and cattle

The Assessment of FSCJ (see ref. 164) described that L-BSE was developed in the cattle that were intracerebrally challenged with the brain homogenate from natural L-BSE cases, and that the symptoms and pathological characteristics of L-BSE cattle seemed to be different from those of classical BSE cases[164]. It has been reported that L-BSE prions can be easily transmitted to wild-type mice and Tg mice over-expressing bovine-, ovine-, and human-PrP by i.c. inoculation. In addition, EFSA has reported that the incubation period and survival time of mice inoculated with L-BSE prions were shorter than those of mice inoculated with classical BSE prions. Based on these findings, EFSA has suggested that pathogenicity of L-BSE prions is likely to be higher than that of classical BSE prions[173].

Infectivity in different tissues from a L-BSE cow identified in Japan (BSE/JP24) has been examined as follows. One ml of 10% medulla oblongata homogenate of BSE/JP24 was intracerebrally inoculated into each of five calves, one of these challenged animals was killed at 10, 12, and 16 mpi for examination of infectivity. To examine the infectivity, the medulla oblongata at the obex, sciatic nerve, brachial plexus vagus nerve, and adrenal gland were collected from the challenged animals at each kill time point, and homogenate of each tissue was intracerebrally inoculated into TgBoPrP Tg mice (five mice per group). As a result, the presence of infectivity was demonstrated in every tissue examined. On the basis of the difference in incubation periods, the infectivity titers of the peripheral nerve such as sciatic nerve and the adrenal gland were estimated to be 1/1000 of that in the medulla oblongata at the level of obex[166].

As described in the section 2 of VI, infectivity of L-BSE has been examined by Tg-mouse bioassay by Suardi *et al*[169]. They examined infectivity in the brain, muscle, kidney, spleen, and lymph node of an asymptomatic L-BSE natural case (14 years old) identified by active surveillance in Italy, as well as of an experimentally-infected L-BSE cow at the terminal stage of disease, using TgbovXV Tg mice (5 to 14 mice per group) inoculated intracerebrally and intraperitoneally with 20 µL and 100 µL, respectively, of 10% homogenate of each tissue. Infection was noted in all the mice exposed to the brain homogenate of either naturally- or experimentally-infected L-BSE case. In addition, clinical signs were observed in one out of the 7 mice challenged with the *gluteus* muscle from the natural L-BSE case, and one out of the 9 mice challenged with *intercostal* muscle from the same case. Clinical signs were also observed in 5 out of the 7 mice inoculated with the *longissimus dorsi* muscle of the experimentally-infected cow[170].

H-BSE prions have been reported to transmit to wild-type mice and Tg mice over-expressing bovine PrP, but the transmission to Tg mice over-expressing human PrP has not been reported[164].

Baron *et al*. has reported that H-BSE PrP^{Sc} was changed to PrP^{Sc} with similar properties as classical BSE PrP^{Sc} after two-generation passage of the brainstem of H-BSE cattle in C57BL/6 wild-type mice. In their experiments, 1% brainstem homogenate (20 µL) each from three H-BSE natural cases and a classical BSE case identified in France was intracerebrally inoculated into mice for a primary passage. The incubation periods after the first and second passage of the H-BSE agent in mice were longer than those of the classical BSE agent. The i.c. deposit of PrP^{Sc} could be observed in 5 out of the 41 mice on the second passage of H-BSE, and WB pattern of PrP^{Sc} in these mice was similar to that of classical BSE. The survival time was 322 to 405 days in mice showing WB pattern of classical BSE, and shorter than 492 to 654 days in mice showing WB pattern of H-BSE. A third passage with the mice brain showing WB pattern of classical BSE caused infection in 15 out of 16 mice with their survival time of 183 ± 6 days, whereas that with the mice

brain showing WB pattern of H-BSE caused infection in 14 out of 16 mice with the survival time of 721 ± 121 days. On the basis of these results, the authors suggested that classical BSE could have originated from sporadic BSE[174].

Torres *et al*. studied the infectivity of four French and one Polish H-BSE natural cases aged 8 to 15 years by bioassays using Tg100 mice **[**Tg mice expressing bovine PrP genes and PrP at the level eight times higher than the i.c. level in cattle**]**. The infectivity of H-BSE was observed in all mice intracerebrally inoculated (20 μL) with 10% brainstem homogenate from these cases. The survival time in the mice was roughly the same as mice inoculated with the brain of classical BSE cattle. The histopathological image of the brain and WB pattern of PrP^{Sc} was similar to those of classical BSE in 3 out of the 12 mice inoculated with the brainstem from one H-BSE case, and in 2 out of the 10 mice inoculated with that from another H-BSE case. WB patterns of PrP^{Sc} were maintained similar to that of classical BSE cattle after serial passages[175].

Pathological and molecular properties of Italian L-BSE, French H-BSE, and UK classical BSE were analyzed by Wilson *et al*. by bioassay using i.c. inoculation with 10% brainstem homogenate (20 μg) of each case to Bov6 Tg mice **[**Tg mice expressing bovine PrP genes**]** (22 or 24 mice per group). PrP^{Sc} was detected by IHC in the brain of mice inoculated with either BSE type. Infection rates in mice inoculated with the brainstem of classical BSE cattle, L-BSE cattle, and H-BSE cattle were 22/22, 24/24, and 17/23, respectively, and PrP^{Sc} accumulation or vacuolar degeneration was observed at 328, 387, and 476 days post-inoculation, respectively. However, the estimated average survival time did not differ among three BSE types. The WB patterns of PrP^{Sc} isolated from the brain of the challenged mice were similar among the BSE types. PrP^{Sc} accumulation was observed also in the spleen. Pathological and molecular properties were also studied using 129/Ola wild-type mice (8 or 24 mice per group) intracerebrally inoculated with brainstem homogenate of each BSE type. All the mice inoculated with the brainstem of classical BSE cattle showed clinical signs with PrP^{Sc} accumulation and vacuolar degeneration in the brain. However, challenge with the L-BSE brainstem caused vacuolation in brain only in one out of the 24 mice, and that with the H-BSE brainstem caused PrP^{Sc} accumulation in brain in only five out of the 24 mice, although none of the inoculated mice showed any clinical signs[176].

Beringue *et al*. studied infectivity of H-BSE isolates by serial i.c. transmission in Tg650 mice over-expressing human PrP (codon 129 MM) **[**One of PrP gene polymorphisms is associated with a substitution of amino acid at codon 129, including methionine/methionine (MM), methionine/valine (MV) and valine/valine (VV) types. Susceptibility of humans to infectious prions is regarded to depend on the polymorphism (See VII. 3) b).**]**. Brain homogenate (equivalent to 2 mg of brain tissue) from three H-BSE bovine cases was inoculated for the primary transmission, and brain homogenate from challenged mice was used for secondary passage. Infection of H-BSE was observed neither in the primary nor in secondary passage[177].

The same research group studied the transmissibility of the BASE, H-BSE, and classical BSE agents into gene-targeted Tg (Tg) mice expressing human PrP. They inoculated intracerebrally 10% brainstem homogenate (20 μg) from BASE, H-BSE, and classical BSE cattle to Tg (HuMM), Tg (HuMV), and Tg (HuVV) mice expressing human PrP (codon 129, MM, MV, VV types of genes), Bov6 Tg mice expressing bovine PrP, and 129/Ola wild-type mice (11 to 29 mice per group). Neurological signs were observed in all the Bov6 Tg mice inoculated with BASE brainstem, but neither PrP^{Sc} accumulation nor vacuolar degeneration was observed in any of Tg (HuMM), Tg (HuMV), and Tg (HuVV) mice when inoculated with brainstem from any types of BSE cattle. From these results, the authors considered that there is an inter-species barrier for transmission between ruminant animals and humans[178].

2) Laboratory studies in primates

Comoy *et al*. studied the transmission of atypical BSE (BASE) from cattle to a primate by i.c. inoculation of 10% homogenate of a mixture of the brainstem and thalamus (25 mg) from an Italian natural BASE (L-BSE) case (15 years old). Transmission of the brainstem (100 mg) from the UK classical BSE cases to two macaques was also examined for comparison. The PrP^{Sc} concentration in the L-BSE brain was one tenth of that in the classical BSE brain. As a result, the incubation period (21 months) and survival time (26 months) of the macaque inoculated with brainstem from the L-BSE case were shorter than those (37.5 months and 40 months) of the macaques inoculated with brainstem of the classical BSE cases. Unlike the macaques infected with classical BSE, vacuolar degeneration and gliosis were widely observed in the cerebrum of the macaque infected with L-BSE. PrP^{Sc} of L-BSE distributed in a diffuse synaptic pattern. The WB pattern of PrP^{Sc} accumulated in macaques was similar to that in the L-BSE case[179].

Transmission of L-BSE to macaques was also studied by Ono *et al*. The brain homogenate (0.2 mL) of a L-BSE case (BSE/JP24) aged 169 months found in Japan was intracerebrally inoculated to two macaques. Both macaques showed neurological signs at 19 to 20 mpi, and were euthanized at 24 to 25 mpi when the animals were at terminal stages of disease. The incubation periods in these macaques were shorter than those reported in macaques[49] inoculated with the classical BSE (C-BSE) agent. PrP^{Sc} distribution was detected mainly in tissues of the CNS. The WB pattern of PrP^{Sc}

Table 15. Number of atypical BSE worldwide (as of December 2010)

Country	H-BSE	L-BSE	Total number
Australia	0	2	2
Canada	1	1	2
Denmark	0	1	1
France	14	13	27
Germany	1	1	2
Ireland	1	0	1
Italy	0	4	4
Japan	0	2	2
Poland	2	8	10
Sweden	1	0	1
Switzerland	1	0	1
Netherlands	1	2	3
UK	3	0	3
US	2	0	2
Total	27	34	61

Ru.G: Summarized by the Department of Epidemiology of Animal Health Institute of Italy **[**The 71st Prion Expert Committee material 3 (29 May 2012) was partially revised.**]**

accumulated in the brain of macaques was similar to that of the inocula from L-BSE case. A diffuse synaptic pattern of PrP^{Sc} in the cerebral cortex was detected by IHC, but also accumulation in the form of fine and coarse granules and/or small plaques were detected in the cerebral cortex and brainstem[180,181].

Mestre-Frances *et al*. examined transmission of atypical BSE to *Microcebus murinus* by i.c. inoculation of 10% brain homogenate equivalent to 5 mg tissues from a French natural L-BSE case, or by oral inoculation of the same homogenate equivalent to 5 or 50 mg tissues,. All four intracerebrally inoculated animals were infected, and showed neurologic symptoms such as slower locomotion, ipsilateral circling behavior and loss of balance. Oral dose of 5 mg induced clinical symptoms similar to those noted in intracerebrally inoculated animals (except ipsilateral circling behavior) in an animal, and minor symptoms in two animals. Oral dose of 50 mg also induced minor symptoms in two animals. PrP^{Sc} was detected by WB in the thalamus and hypothalamus of all animals showing clinical symptoms, except in an animal orally administered with 5 mg[182].

4. Epidemiological Characteristics of Atypical BSE

Exact number of atypical BSE cases, and their frequency and distribution in the world are unknown at present, because discrimination between atypical BSE and classical BSE has not been required in the report to the OIE[164].

The 61 cases of atypical BSE reported worldwide up to December 2010 are summarized in **Table 15**. In addition to these cases, H-BSE was detected in a zebu aged 19 years at a Swedish zoo[183] **[**This zebu is the only case of atypical BSE in which clinical symptoms were observed. With the exception of the above, no clear clinical symptoms of BSE have been observed in atypical BSE cases.**]**.

Biacabe *et al*. studied the epidemiological situation of BSE cases from 2001 to 2007 in France. In France, active surveillance was conducted from July 2001 to July 2007 according to the EU surveillance programme, whereby 17.12 million adult cattle were tested for BSE, accounting for 30% of cattle tested in the EU. Out of the 17.12 million cattle, approximately 3.6 million cattle were aged 8 years or older. Through the surveillance, 645 cattle were determined to be BSE positive, of which 584 were classical BSE, 7 were H-BSE, 6 were L-BSE, and 48 were uncertain, by WB analysis.

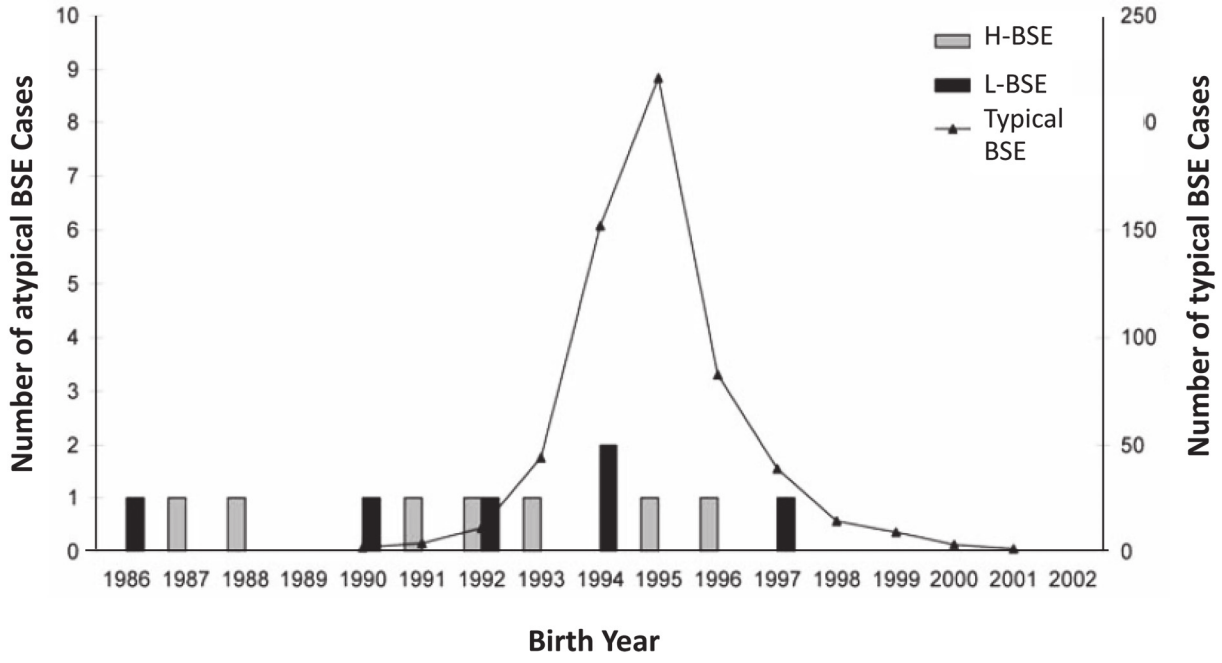


Fig.10. Distribution of classical BSE cases and atypical BSE cases in France by birth cohort based on the literatures of Biacabe *et al*.[184].

All the atypical BSE cattle found by the active surveillance were over 8 years old, of which 9 were fallen stock and 4 were healthy slaughtered cattle. The birth year of H-BSE and L-BSE cattle distributed ubiquitously in 1986–1997.

In contrast, the birth year of classical BSE cattle was restricted in 1990–2001 (refer to **Fig. 10**), showing that the distribution of the birth cohort and the frequency of atypical BSE cases are distinct from those of classical BSE cases. The authors considered that the findings are consistent with the hypothesis that atypical BSE might be a sporadic prion disease, although the possible transmission through feed can not be excluded[184].

Passive surveillance by EU on diseased cattle failed to detect atypical BSE[173], suggesting that the signs of atypical BSE may differ from those of classical BSE. In addition, given that the prevalence of atypical BSE was one out of 3 million in France and Germany, the sample size of passive surveillance was suggested to be too small to detect atypical BSE which mainly occur in old cattle[185].

Polak *et al*. confirmed six H-BSE and one L-BSE cases by retrospectively analyzing 50 BSE cases that had been identified in the active surveillance in Poland from 2002 to 2006[186]. Tester *et al*. found one H-BSE case by examining brain tissues from 37 BSE cases aged 8 years or older identified in the passive surveillance in Switzerland[187]. Dudas *et al*. found one H-BSE and L-BSE case each by retrospectively analyzing the past 17 BSE cases identified in the active surveillance in Canada from 2003 to 2009[188]. Dobly *et al*. retrospectively examined 42 BSE cases aged seven years or older detected in Belgium from 1999 to 2008, and found no atypical BSE cases[189].

No atypical BSE case was identified in the 523 BSE cases detected in the passive surveillance in the UK[190]. Apart from the result, three H-BSE cases have been reported[191,192]. Other than the above, atypical BSE cases have been reported in Denmark, Germany, Ireland, the Netherlands, Sweden, the United State, Japan, etc.[185]

Sala *et al*. analyzed the characteristics of 12 L-BSE and 11 H-BSE cases detected in France from January 2001 to late 2009 in comparison with those of classical BSE cases detected during the same period. The L-BSE and H-BSE cases were identified at the age of 8.4 to 18.7 years (average 12.4 years) and, 8.3 to 18.2 years (average 12.5 years), respectively, whereas classical BSE cases were at the age of 3.5 to 15.4 years (average 7.0 years). A spatial scan statistical analysis on each atypical BSE case resulted in the distribution of four L-BSE cases in an oval area with 32 km of the major axis and 12 km of the minor axis in mid-west France, and they concluded that this cluster area was a geographically significant cluster of L-BSE cases[193].

5. Summary

1) Properties and distribution of atypical BSE prions in cattle

Distribution of atypical BSE prions in cattle has not fully been understood.

Unlike in classical BSE, relatively high concentrations of PrP^{Sc} have been detected in the thalamus, olfactory bulb, and frontal lobe rather than in the obex and brainstem in L-BSE cattle. In addition, plaques are occasionally accompanied in L-BSE cases. Intracerebral distribution of PrP^{Sc} in L-BSE cases was found to show a different pattern from that of classical BSE. At present, PrP^{Sc} distribution in H-BSE cattle has been reported only in the CNS.

2) Transmissibility of atypical BSE prions

In laboratory studies with serial passages of atypical BSE prions in mice, some of the mice presented pathological features similar to those of classical BSE, but the interrelationship among classical BSE, H-BSE and L-BSE prions is not clearly understood.

L-BSE prions were easily transmitted to Tg mice over-expressing human PrP (codon 129 MM), but not to gene-targeted Tg mice expressing human PrP (codon 129 MM). H-BSE prions, however, transmitted to neither the over-expressing Tg mice nor the gene-targeted Tg mice.

L-BSE prions are more transmissible to macaques than classical BSE prions. Infection was observed in *Microcebus murinus* when orally inoculated with L-BSE prions. Primates are therefore considered susceptible to L-BSE prions.

These findings suggest a potential of L-BSE prions as a zoonotic pathogen, and therefore it cannot be excluded that humans could be infected with atypical BSE prions . However, there may be a very high interspecies barrier against transmission of H-BSE from cattle to humans.

3) Number of cases and epidemiological characteristics of atypical BSE

Atypical BSE is classified into H-BSE and L-BSE, and most cases are detected in cattle at the age of over 8 years (ranging from 6.3 to 18 years). The number of atypical BSE cases detected in the whole world is 61, as of December 2010. The prevalence of H-BSE and L-BSE detected under the EU surveillance program in France (2001 to 2007) are 0.41 and 0.35, respectively, out of one million cattle of the age over 30 months. Within the animals of the age over 8 years, the prevalence of H-BSE and L-BSE was 1.9 and 1.7, respectively, out of one million animals.

The atypical BSE case (BSE/JP8) of 23 months of age in Japan was found among 13.7 million cattle tested for BSE including fallen stock. In the medulla oblongata of this case, the amount of PrP^{Sc} was roughly 1/1000 of that of classical BSE cattle, and no infectivity was observed by the infection study. The frequency and distribution of atypical BSE in the world are unknown at present, because discrimination between atypical and classical BSE is not required for the report to the OIE[164]. Furthermore, the origin of atypical BSE is unknown, and thus it cannot be excluded that atypical BSE might be transmitted through feed as is classical BSE. However, based on birth cohorts and frequency of identified atypical BSE cases, it has also been suggested that atypical BSE could be a sporadic prion disease.

VII. Variant Creutzfeldt-Jakob Disease (vCJD)

1. Prevalence and Epidemiology of vCJD

1) Background of vCJD

vCJD is one of human TSE [CJD is, from its prevalence mechanism, classified into the three categories: “sporadic CJD (sCJD)”, “heritable CJD” and “acquired CJD”. vCJD belongs to “acquired CJD”.]. Although no direct scientific evidence is confirmed at present, this disease is considered to be a zoonosis that may be transmitted to humans through food derived from BSE cattle, based on the following findings[173,194–196]: Infection has been confirmed in mice experimentally inoculated with the brain tissues from BSE cattle and vCJD patients; molecular and biological properties of the BSE agent was similar to those of the vCJD agent; and there was a correlation between chronological changes of occurrence of BSE and those of vCJD. The prevalence of BSE has decreased due to various control measures against TSE of livestock, following which the occurrence of vCJD has also decreased (**Fig. 11**)[173,196,197].

In the UK, the use of specified organs (specified bovine offal (SBO): brain, spinal cord, spleen, thymus, and tonsils) for food was prohibited in November 1989 as food safety measures against BSE. MRM from the skull and that from vertebral column of cattle were prohibited from the use for food in 1992 and 1995, respectively. In addition, the use of cattle over 30 months of age as food was prohibited in 1996 (lifted in September 2005)[198].

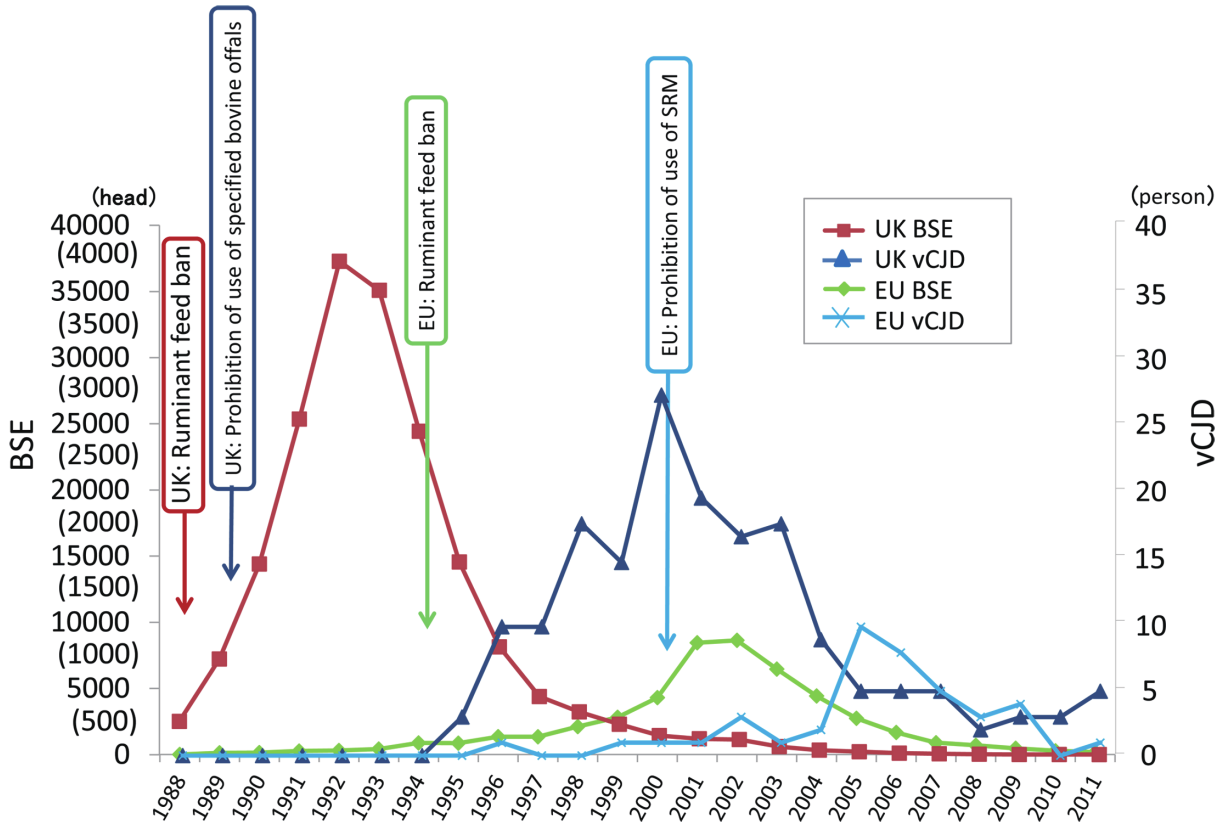


Fig.11. Changes in the number of reported BSE and vCJD cases in the UK and EU from 1988 to 2008
Note) The number of the left and right vertical axes indicates the annual number of BSE and vCJD cases, respectively (the number of BSE cases in EU is shown in parenthesis on left vertical axis).
Based on vCJD factsheet (European Centre for Disease Prevention and Control (ECDC) [http://www.ecdc.europa.eu/es/healthtopics/Pages/3604_Factsheet.aspx.].

In the EU, since October 2000, according to EC Legislation 418/2000, the removal and disposal of tissues possibly containing SRMs have been regulated, and the tissues is prohibited from the use for food[37].

In Japan, the Ordinance for Enforcement of the Slaughterhouse Act was amended in October 2001. According to the amendment, skulls excluding cheek meat and tongues, spinal cords, and distal ileum [two meters from the junction to the caecum] of all cattle are obliged to be removed and incinerated at the time of slaughtering, and the use of these tissues for food is prohibited[11].

In European nations, the surveillance of CJD cases started in 1993. Since then, the member states joining this surveillance have increased and continued the surveillance still now[173].

The surveillance of prion disease including vCJD started in Japan in fiscal year 1996. In April 1999, along with the enforcement of Act on Prevention of Infectious Diseases and Medical Care for Patients Suffering Infectious Diseases (Act No. 114 of 1998), a system was constructed to ascertain all CJDs in Japan as notifiable infectious diseases.[199,200].

In the United States, the Centers for Disease Control and Prevention (CDC) ascertains the trend and latest incidence rate of vCJDs in the country, using multiple surveillance mechanisms. Medical doctors are encouraged to report suspected vCJD cases to a State Health Department through a Regional Health Department. CDC conducts surveys on cause-of-death data, or on clinical and neuropathological records of fatal vCJD cases aged less than 55 years reported by medical professionals[201].

In Canada, the CJD surveillance system was established in 1998. According to the state law, medical doctors must report CJDs to Regional Health Authorities. Thereafter, officials stationed nationwide are engaged in investigation of medical records[202].

2) Number of vCJD patients in the world

There are 227 patients of vCJD [The number of patients definitely diagnosed as vCJD by pathological examination.] in total in the world (as of July 2012) [The National Creutzfeldt-Jakob Disease Research & Surveillance Unit

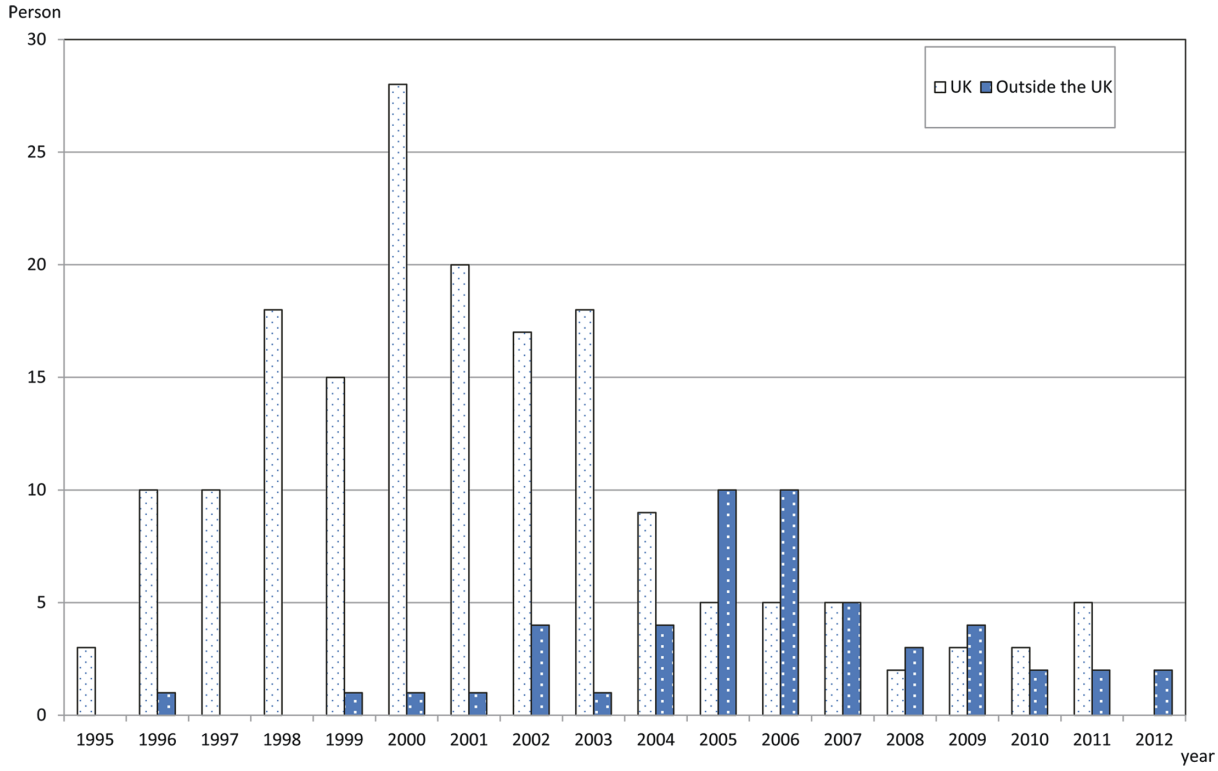


Fig.12. Annual number of vCJD patients

(NCJDRSU) http://www.cjd.ed.ac.uk/vcjdworld.htm.**]**, and 176 out of them were confirmed in the UK. The number of patients confirmed in the other countries are; 27 in France, 4 in Ireland, 2 in Italy, 3 in the Netherlands, 2 in Portugal, 5 in Spain, 3 in the UA, 2 in Canada, 1 in Saudi Arabia, 1 in Taiwan, and 1 in Japan (**Fig. 12**).

a) Incidence of vCJD in the UK

In the UK, the first vCJD patient was identified in 1995. Thereafter, the annual number of reported patients increased to peak of 28 in 2000, and has been decreasing to two to five since 2005. After the use of SBO as food was prohibited in 1989, no vCJD patient has been identified in the population born after 1990. As of July 2012, the total number of vCJD patients in the UK is 176, and there are no surviving patients[203,204].

As for the other countries than the UK, a vCJD case was first identified in France in 1996. Since then, the number of the patients in these countries peaked in 2005 and 2006, and a total of 51 patients have been confirmed from 1999 to present **[**The European Creutzfeldt Jakob Disease Surveillance Network. http://www.eurocjd.ed.ac.uk/surveillance%20data%204.htm.**]**. Cattle imported from the UK have been suggested to be the most important source of exposure to humans in countries outside the UK, because the number of patients of vCJD confirmed in each country was related to the number of live cattle imported from the UK from 1980 to 1990 and the amount of dressed carcasses imported from the UK from 1980 to 1996[205].

Since the discovery of BSE in 1986, over 180,000 cattle in the UK developed the disease, and one million infected cattle were estimated to have entered into the food chain from 1985 to 1996[206]. Although the total number of vCJD patients in the UK had been estimated to be 5,000 at worst[15], the confirmed number of the patients in the UK is 176 as of July 2012, including three cases of infection by blood transfusion **[**The National Creutzfeldt-Jakob Disease Research & Surveillance Unit (NCJDRSU) http://www.cjd.ed.ac.uk/vcjdworld.htm.**]**.

b) Incidence of vCJD in Japan

According to the survey of infectious diseases in Japan by MHLW and surveillance by study groups, only one vCJD case was reported in February 2005 as of July 2012 in Japan. The patient was a male, stayed in the UK, France, and Spain temporarily (approximately for one month in total) in February 1990 at the age of 37, and had no history of surgery such as dura mater transplant. In June 2001 (age 48), the patient had difficulty in writing, subsequently presented mental disturbance and perception disorder, reached akinetic mutism, and died in December 2004 at the age of 51. Examination by MRI and electroencephalography (EEG) while alive diagnosed him sCJD. However, pathological examination (his-

Table 16. Clinical differences between sCJD and vCJD

	sCJD	vCJD
Mean age of death (years)	67	29
Mean duration of illness (months)	4	13
Rapidly progressive dementia	Common	Rare
Psychiatric symptoms at onset	Rare	Common
Sensory disorder	Rare	Common

tological observation and IHC) of the post-mortem brain and WB definitely diagnosed him vCJD. The clinical course of this case was approximately 43 months. The genotype of PrP codon 129 of this patient was MM type[207]. MHLW concluded that "although the possibility of the patient being infected in France or Japan cannot be excluded, it is highly probable that he was infected in the UK"[208] based on investigation of the infection route.

3) Epidemiology of vCJD

a) Onset age and incubation period of vCJD

vCJD is different from sCJD in age of onset and pathological characteristics. Clinical differences between sCJD and vCJD are shown in **Table 16**[194].

Little is known about the incubation period of vCJD, and a wide range has been suggested as estimates such as from a few years to over 25 years, under various hypotheses[15]. Since the late 1990s, a few cases of PrP^{sc} accumulation in the spleen, appendix, and tonsils without clinical signs have been reported, indicating that there could be a human population in the stage prior to onset of the disease after being infected with the BSE agent[209–211]. However, the details on such a population are unknown, and therefore it is difficult to predict the incubation period in them[173].

b) Genetic characteristics related to the infection of vCJD

PrP gene polymorphism includes MM, MV and VV types in the 129th amino acid, and these amino acid types have been suggested to relate with the risk of development of vCJD. The genotype of patients suffering from vCJD that has been reported so far in the UK is MM type, and humans of this genotype are considered to have a shorter incubation period of vCJD and/or higher susceptibility than those of other genotypes[173]. The proportion of MM type in the whole population in Japan has been reported to be 91.6%, which is higher than in the UK[15].

In 2004, Peden *et al*. reported that PrP^{Sc} was detected in the spleen of an elderly patient of MV type in the UK. No nervous disorder was confirmed in this patient, and PrP^{sc} was not detected in the brain and spinal cord. Since the patient had a history of blood transfusion from a vCJD patient, the authors considered that development of vCJD by infection through human-to-human blood transfusion is not limited to MM type[212]. In 2009, Kaski *et al*. reported a 30 year-old male patient of vCJD whose PrP gene codon 129 was MV type, but the patient had no history of blood transfusion and tissue transplant. The autopsy data of the patient has not been reported[213]. Generally, human TSE occurs regardless of amino acid polymorphisms at prion gene codon 129. In Kuru, MV type has been reported to have a long incubation period to onset. Therefore, if it is assumed that the incubation period of vCJD in populations with MV and VV types is as long as that of Kuru, vCJD patients of MV and VV type with long incubation periods could be found in future[173,194,212].

c) Accumulation of PrP in the human appendix and tonsil

Hilton *et al*. examined accumulation of PrP by IHC in the appendices and tonsils excised from 8,318 UK people aged 10 to 50 years from 1995 to 1999. As a result, the accumulation of PrP was confirmed in one lymph follicle of an appendix sample[209]. Furthermore, the accumulation of PrP was confirmed by IHC in three samples including the above sample among 12,674 appendix and tonsil samples excised after 1995 (the majority of the samples were appendices excised from people aged 20 to 29 years)[214]. On the other hand, the accumulation of PrP was not detected in 2000 samples of tonsil excised in London area from 2000 to 2002 by Frosh *et al*. using immunological method, nor in 63,007 samples excised in the period from 2004 to 2008 by Clewley *et al*[211,215].

Wadsworth *et al*. studied the presence of infectivity of the brain, spleen, and appendix from vCJD patients, and the appendix from two cases of codon 129 VV type of the above three cases where the accumulation of PrP was reported by Hilton *et al*.[214]. They examined the infectivity by inoculating the tissue homogenate (0.2% to 1%) into the brain of

human PrP (codon 129 MM type) Tg mice. Infectivity was confirmed in the mice inoculated with the brain homogenate, but not in those inoculated with homogenate of the spleen and appendix tissues[216]. So far, no report has indicated the presence of infectivity in the appendix and spleen where the accumulation of PrP was detected.

2. Risk of BSE Prion Infection in Humans

1) Interspecies barrier between cattle and humans

The interspecies barrier between cattle and humans has been suggested based on the results of experimental studies on infectivity of BSE prions using different animal species. Up to now there is no finding to exclude the possibility of the interspecies barrier.

Comer *et al*. estimated the level of exposure to humans when the infected animals were consumed as food. The total bovine oral ID_{50} units per one fully infected bovine slaughtered for consumption from 1980 to 2001 was estimated based on the estimates of infectivity titer of cattle tissues and exposure route to humans. Bovine oral ID_{50} units per one fully infected bovine was, compared to the peak in 1982, estimated to be approximately 1/10 after the use of SBO in food was prohibited in 1989, and approximately 1/100 in 2001. Furthermore, bovine oral ID_{50} unit of each year from 1980 to 2009 in the UK was estimated by back-calculation method of Ferguson *et al*. Based on the estimates, the infectivity incorporated into human food from 1980 to 2001 in the UK was calculated to be 54,000,000 bovine oral ID_{50} units, and 99.4% of it was estimated to be from cattle aged over 30 months. The authors considered this result to indicate the effectiveness of food ban on cattle aged over 30 months[35,217].

In the risk assessment of gelatin in 2006, the EFSA estimated the infectivity titer incorporated into human food to be 5,000,000 bovine oral ID_{50}, or 0.004 bovine oral ID_{50}/person/year (60,000,000 people for 20 years), pointing out that Comer *et al*. overestimated by 10-fold the infectivity titer of CNS. Based on this figure and the report that the number of vCJD patients was 550 at most[218], EFSA estimated the susceptibility of humans whose prion gene codon 129 is MM type to be 1/4000 of that of cattle[219].

2) Infectivity of BSE prion in Tg mice over-expressing human PrP

Tg mice over-expressing various human PrPs have been developed[220,221] to study infection risks of BSE prions in humans.

Asante *et al*. conducted experimental studies using three types of Tg mice expressing PrP four to six times higher than normal human brains, namely 129MM Tg45, 129VV Tg152, and 129MV Tg45/152 mice. I.c. inoculation of 30 μL of 1% homogenate of either the brain from vCJD patients or the brainstem from naturally infected BSE cattle caused vCJD-like neuropathological changes in the brain in 129 MM Tg45 mice. Similarly, 129MV Tg 45/152 mice were intracerebrally challenged with 1% homogenate of each tissue. Clinical signs, and WB and IHC results were positive in all the mice inoculated with the brain homogenate from vCJD patients. On the other hand, in mice inoculated with the brainstem of BSE cattle, the IHC results were all negative, and clinical signs and/or WB results were positive only in 12 out of 41 mice. The findings showed the barrier against bovine-to-human transmission of BSE prions in human 129MV type PrP Tg mice[222,223].

Wadsworth *et al*. inoculated the brain tissues from BSE cattle and those from vCJD patients into three lines of Tg mice over-expressing human PrP, namely 129MM Tg35, 129MM Tg45, and 129VV Tg152 mice. As a result, when challenged with the brain from BSE cattle, infection was confirmed in either mouse line, with the rate of infection of 9/12 (75%), 14/49 (29%), and 10/26 (39%) in 129MM Tg35, 129MM Tg45, and 129VV Tg152, respectively. Although clinical signs were confirmed in 129VV Tg152 mice, PrP^{Sc} could not be detected in their brain. In addition, the second passage in 129VV Tg152 mice failed to induce infection (0/27). The rates of infection in 129MM Tg45, 129MM Tg 35 and 129VV Tg152 mice inoculated with the brain from vCJD patients were 4/4 (100%), 14/14 (100%), and 25/56 (45%), respectively. The second passage of the brain homogenate from infected 129VV Tg152 mice into 129VV Tg152 and 129MM Tg35 mice caused infection in both Tg mice. Moreover, the rate of infection was higher in 129MM Tg35 mice with 14/15 (93%) than in 129VVTg152 mice with 7/11 (64%). From these findings, the authors considered that the interspecies barrier of BSE prions is higher in mice expressing human PrP 129VV than in those expressing human PrP 129MM[224].

In order to examine interspecies barriers, Bishop *et al*. intracerebrally inoculated 0.02 mL each of brain homogenates from BSE cattle and from vCJD patients into Tg mice over-expressing bovine PrP, and gene-targeted Tg mice (18 to 23 mice/group) expressing human PrP of different amino acid types (HuMM, HuMV, and HuVV Tg mice). The brain homogenate from BSE cattle infected Tg mice over-expressing bovine PrP (22/22) but not any human PrP Tg mice. The rates of infection in mice challenged with the brain homogenates from vCJD patients were 11/17 (65%), 11/16 (69%), and

1/16 (6%) in HuMM, HuMV, and HuVV Tg mice, respectively. Hence, the authors considered that interspecies barriers clearly exist between cattle and humans[225].

3) Infectivity of classical BSE prions in primates

PrP gene codon 129 of cynomolgus macaques is MM and the animals show pathological symptoms similar to that of human vCJD when inoculated with BSE prions.

Lasmézas *et al*. studied infectivity of classical BSE prions with serial passages in cynomolgus macaques. For the first passage, 400 µL of 25% brain homogenate from BSE cattle was intracerebrally inoculated to cynomolgus macaques. The incubation periods in three monkeys of the first-passage were 36, 40, and 40 months. On the other hand, the incubation periods in the two animals of second passage were shortened to 18 and 20 months[226,227]. Furthermore, the same authors orally administered or intravenously inoculated the brain from these cynomolgus macaques to other cynomolgus macaques (orally to two monkeys; intravenously to one monkey) to examine the transmissibility and distribution of BSE prions. The two monkeys orally administered with 5 g of the brain reached a terminal stage of clinical signs at 47 and 51 months post-administration and were then euthanized. The monkeys intravenously inoculated with 40 mg, 4 mg, and 0.4 mg of the brain homogenates reached a terminal stage of clinical signs at 25, 38, and 33 mpi, respectively, and then they were euthanized. Tissue distribution of PrP^{Sc} in the infected animals indicated the presence of PrP^{Sc} in all the intestines from the duodenum to rectum, and in lymphatic tissues such as the spleen and tonsils, regardless of infection route. PrP^{Sc} was found in Peyer's patches, and in the enteric nervous system and nerve fibers of intestinal mucosa. The amount of PrP^{Sc} accumulated in tonsils was equal to or more than 10% of that in brain in intravenously infected animals and 1 to 10% of that in orally infected animals, but the amount in other tissues was 0.02 to 4% of that in brain[228].

Lasmézas *et al*. inoculated orally 5 g of brain homogenates from BSE cattle to two cynomolgus macaques of 4 years old. As a result, one of them developed clinical signs at 60 months post-administration and was then enthanized at 63 months. The other animal did not show the clinical signs even at 76 months, and PrP^{Sc} was not detected in the biopsy of the tonsils conducted at 72 months post-administration. By comparing with the results of bovine oral administration studies using the inocula of the same concentration, the authors estimated that the susceptibility to oral exposure of primates is 1/7 to 1/20 of that of cattle[229].

Herzog *et al*. studied tissue distribution of PrP^{Sc} in cynomolgus macaques received i.c. inoculation of 400 µL of 10% brain homogenates from vCJD patients, intra-tonsil inoculation of 80 µL of the same homogenate, and oral administration of 5 g of brain tissue from BSE cattle. The results showed that the amount of PrP^{Sc} accumulated in the spleen was approximately 4% of that in brain, regardless of administered brain samples and administration routes. In intestinal Peyer's patches the maximum PrP^{Sc} accumulation was found in animals received oral administration of the BSE brains, but the amount was approximately 0.1% of that found in brain. The amounts of accumulation in the tongues and muscles were approximately 1/5,000 and 1/10,000 to 1/20,000, respectively, of that found in brain regardless of administered brain samples and administration routes[230].

Ono *et al*. studied infectivity of BSE prions by intracerebrally inoculating 200 µL of 10% BSE cattle brain homogenates into three cynomolgus macaques. The animals developed neuronal symptoms 27 to 44 months after inoculation, and were euthanized 8 to 15 months later. Furthermore, they conducted the second passage experiment where a brain homogenate of one cynomolgus macaque that developed symptoms at 29 months was inoculated intracerebrally into two other cynomolgus macaques. In these macaques, the incubation period was markedly shortened to 13 and 15 months. Histopathological images of the brains of the cynomolgus macaques were similar to those of vCJD, and WB patterns were identical with those of BSE PrP^{Sc}. The accumulation of PrP^{Sc} was mainly observed in the CNS but not in lymphatic tissues such as the tonsils, spleen, and appendix[231].

3. Summary

1) Prevalence situation of vCJD

Until July 2012, 227 cases of vCJD were reported in the whole world. The incidence of vCJD in the UK strongly indicated its epidemiological relation to the occurrence of BSE. On the other hand, the annual number of identified vCJD cases in the UK reached a peak with 28 in 2000, and has decreased to 2 to 5 since 2005. The use of SBO in food was prohibited in 1989, and no vCJD patient has been confirmed in populations born from 1990 to date. This is attributable to the comprehensive effects of BSE countermeasures. As in "Measures against Bovine Spongiform Encephalopathy (BSE) in Japan −Interim Report- (September 2004)"[15], the regulation of animal feed is regarded as a fundamental measure to prevent BSE infection and to guarantee the reduction of risk of BSE prion infection from cattle to humans.

In the UK, the first vCJD patient was documented in 1995, and as of July 2012 the total number of vCJD cases is 176.

Case of vCJD in Japan as of July 2012 is only one that reported in February 2005. Regarding the cause of this case, it was concluded that "although the possibility that the patient was infected in France or Japan cannot be excluded, he was highly probable to have been infected in the UK."

The "Interim Report on Measures against Bovine Spongiform Encephalopathy (BSE) in Japan" issued in September 2004[15] predicted the number of vCJD cases that will be caused by domestic beef and beef offal in Japan to be 0.1 to 0.9, predicting the number of vCJD cases in the UK to be 5,000 patients at worst. However, the vCJD occurrence in the UK is 176 patients to date, which is only 3.5% of the prediction, and the number of BSE cattle has been remarkably decreasing. Therefore, it is clear that the incidence of vCJD will not exceed the figures predicted in the "Interim Report."

2) Epidemiology of vCJD

Little is known about the incubation period of vCJD, and estimates with a wide range have been suggested, such as from a few years to over 25 years, in different hypotheses.

The amino-acid polymorphism (codon 129) of the prion gene codon 129 of vCJD patients so far reported in the UK is MM type, and incubation period of vCJD in peoples with this genotype has been considered shorter, or peoples with this genotype are considered to be more susceptible, or both, compared to peoples with other genotypes. Assuming that the incubation period of vCJD is as long as that of Kuru, although the precise relationship between amino acid polymorphisms at prion gene codon 129 and the incubation period of vCJD remains uncertain, vCJD patients of MV and VV types with long incubation periods might be identified in future. Therefore, it is important to continue monitoring the incidence of vCJD by appropriate surveillance.

3) Risk of BSE prion infection in humans

The risk of BSE prion infection in humans has been studied by the experiment of i.c., intravenous, or oral exposure of Tg mice over-expressing human PrP or primates close to humans to BSE prion containing tissues. Results from these studies are summarized below.

The susceptibility of Tg mice over-expressing human PrP to BSE prion is known to depend on the genotype. For instance, the results of BSE prion inoculation experiments in Tg mice carrying human prion gene codon 129 MM, MV, and VV genotypes have been reported. As a control experiment, vCJD prions were inoculated and observed up to onset of the disease. The results showed that the interspecies barrier of BSE prions in Tg mice over-expressing human PrP was higher than the barrier of vCJD prion, and that BSE prions readily infected the Tg mice of MM and MV genotypes, but not readily infect the mice of VV genotype.

When primates were intracerebrally inoculated as the first passage with the brain homogenates from BSE cattle, and subsequently the brain homogenates from these infected primates were intracerebrally inoculated to the other primates as the second passage, the second passage shortened the incubation period. The pathological changes in the primate brain were similar to those caused by vCJD. Oral administration experiments have suggested that the susceptibility to BSE prions is lower in primates than in cattle. These results indicate the presence of interspecies barrier of BSE prion between cattle and primates.

VIII. Risk Assessment

Human health risks associated with BSE were assessed in response to the requests from MHLW. In the process of risk assessment, FSCJ utilized reference documents provided by MHLW regarding BSE situation as well as BSE literatures collected so far through the FSCJ's work on risk assessment. FSCJ decided to assess at first 1) the domestic measures and 2) the border measures, prior to the assessment on the other requested issue, 3) the influence of additional changes of domestic and/or border measures.

1. The BSE Status

The total number of BSE cattle reported worldwide until July 2012 is 190,629. The number of BSE cases peaked with 37,316 in 1992, and fell to 45 cases in 2010 and 29 cases in 2011. MHLW requested the assessment for the above 5 countries, namely Japan, the United States, Canada, France, and the Netherlands.

In Japan, 36 cases of BSE cattle were confirmed as of July 2012, and two cases among them were atypical BSE. The last born case was found in the cohort of January 2002, and no BSE case has been found in the later birth cohorts.

In the US, four BSE cases were confirmed until July 2012. One of them was imported from Canada, while other three were atypical BSE. No BSE incidence by cohort has been confirmed after one case born in September 2001.

In Canada, except for one case confirmed in the US, 19 BSE cases were confirmed until July 2012. Of the 19, one was imported from the UK and two cases were atypical BSE. No BSE has been confirmed in birth cohorts after one case born in August 2004.

In France, 1,023 BSE cases were confirmed until July 2012. Of them, 27 cases were atypical BSE (as of December 2010). No BSE incidence by cohort has been confirmed after one case born in April 2004.

In the Netherlands, 88 cases of BSE were confirmed as of July 2012. Of the 88, four cases were atypical BSE. No BSE incidence by cohort has been confirmed after one case born in February 2001.

The assessment shows that no BSE case has been confirmed in the five countries for the recent eight years after one born in August 2004.

2. Feed Control Measures in Each Country and Their Effects

In Japan and the other four countries assessed, feed control measures including voluntary ban were introduced by 1997 to prevent BSE-prion contaminated tissues from entering the feed supply, and since then, the measures have been extended gradually to include the prevention of cross-contamination.

In Japan, uses of all mammal-derived proteins for ruminant feed and ruminant-derived proteins for feed of livestock other than ruminants were prohibited in October 2001.

The US prohibited the use of the brain and spinal cord of cattle aged over 30 months for every livestock feed and pet food in October 2009.

Canada prohibited the use of SRMs (the skull, brain, trigeminal ganglia, eyes, tonsils, spinal cord, and dorsal root ganglia of cattle aged 30 months or older, and the distal ileum of cattle of all ages) in every livestock feed and pet food in July 2007.

France banned the use of all animal-derived proteins for all livestock feed in November 2000.

The Netherlands banned the use of all animal proteins for all livestock feed in December 2000.

At least 35 months (as of September 2012) have passed since feed control measures including preventive measures against cross-contamination were strengthened in the five assessed countries.

The surveillance level in Japan and the other four countries are equal or above the level capable of detecting BSE at least one per 100,000 in adult cattle indicated by the OIE point system for “countries with controlled risks”. In the five countries, with the exception of one case in Japan, three in France, and one in the Netherlands, no BSE cases were identified after the feed control measures were strengthened. Therefore, the feed control measures taken in these countries are markedly effective in reducing BSE.

3. SRM and Meat Processing Process

In Japan and the other four countries, SRMs (except tonsils in Canada) are defined based on or beyond the OIE recommendations for the importation of cattle from a country with controlled BSE risk. In these countries, prohibition of pithing as well as the removal of SRMs are implemented as risk reduction measures in meat processing processes.

The risk of exposure of cattle to BSE prions was reduced by the introduction of BSE measures including feed restriction. This outcome, together with the introduction of the risk reduction measures in meat processing processes, contributes to the extremely low level of the risk of exposure of human to the BSE prions in Japan and other four countries.

4. Infection Experiments in Cattle

Infection experiments in cattle suggested an inverse relationship between the dose of BSE prions and incubation period, where oral administration of the brainstem from the UK BSE cattle at the dose of 100 g, 10 g, 1 g, or 100 mg resulted in the clinical onsets in the challenged cattle with mean incubation periods of 31, 41, 45, or 53 months, respectively. Incidence was very low with doses less than 100 mg.

In brains of cattle orally administered at their ages of four to six months with 1 g of the brainstem of the UK BSE cattle, PrP^{Sc} was not detected up to 42 months, but was detected at 44 months post-administration or later. In an oral infection experiment using 5 g of the brainstem into cattle of 4 months old in Japan, PrP^{Sc} is not detected up to 30 months in the CNS including the brain and spinal cord, but detected at 34 months post-administration or later. In another infec-

tion experiment, PrP^{Sc} was also detected in the thoracic spinal cord, but not in the medulla oblongata at the level of obex, at 48 months post-administration. When brain tissues from BSE cattle were inoculated intracerebrally to calves to verify the time point of the accumulation of PrP^{Sc}, the earliest time point of detection of PrP^{Sc} in the CNS was 7 to 8 months before the onset of clinical symptoms.

For tissues of the spinal cord and medulla derived from cattle inoculated with excess amounts of the BSE case-derived brainstem (100 g), a bioassay using Tg mice over-expressing bovine PrP was performed. The result obtained suggests the earlier appearance of the infectivity in the cattle thoracic spinal cord than in the medulla oblongata. The oral dose of 100 g in cattle is, however, considered to be too high to occur through feed under the natural infecting situations.

Incubation period, when a large number of BSE cases were confirmed in the UK, has been estimated to be 5 to 5.5 years on average on the basis of the prevalence situation in the field.

Experiments described above, thus, suggest that the average amount of BSE prions possibly ingested in the field in the UK has been estimated to be equivalent to 100 mg to 1 g of BSE cattle brainstem used in the oral infection studies. It was the time when feed was most heavily contaminated with BSE prions in the UK.

The amount of PrP^{Sc} accumulated in the medulla oblongata at the level of obex of Japanese BSE positive case (BSE/JP9) aged 21 months was estimated to be approximately 1/1,000 of that of classical BSE-infected cattle. The infectivity was not detected in tissues of this case in experiments using Tg mice over-expressing bovine PrP, and therefore considered to be negligible in humans.

5. vCJD

Total 227 cases of vCJD have been reported in the world until 2012, but the reported number of the annual incidence was already decreasing after attaining its peak. This is likely due to the effectiveness of BSE countermeasures. In the UK where the huge number of vCJD cases has been recorded, the incidence decreased after the peak in 2000 through the prohibition of the use of SRM in food since 1989. None of vCJD patients has been confirmed to date among the population born in 1990 or later. FSCJ reconfirmed, as mentioned in "Measures against Bovine Spongiform Encephalopathy (BSE) in Japan −Interim Report- (Issued in September 2004)", that the regulatory measures on animal feed are essential to prevent BSE and also to reduce the risk of human infection with BSE prions.

In the Interim Report, beef and beef offal produced domestically in Japan are expected to cause 0.1 to 0.9 patients of vCJD. This report also predicted to cause 5000 vCJD patients in worst scenario in the UK. The actual numbers of vCJD patients reported in the UK are, however, 176 to date, which correspond to 3.5% of the prediction. In addition, the numbers of BSE cattle in both countries are markedly decreased. Therefore, FSCJ concludes that the outbreak will not exceed these figures predicted in the 2004 report.

From the results of laboratory studies using Tg mice and monkeys over-expressing human PrP, human is less susceptible to BSE prions than is bovine, due to the interspecies barrier between bovine and humans.

6. Atypical BSE

The possibility of infection of atypical BSE prions to humans cannot be excluded, because infection to primates has been observed in i.c. inoculation experiments.

Atypical BSE is regarded to occur rarely in the old cattle, because only 61 cattle of atypical BSE were confirmed worldwide as of December 2010 mostly at their ages of eight years or over (age range at the time of confirmation was 6.3 to 18 years). To date, approximately 13.7 million of cattle including dead cattle have been examined for BSE in Japan, and two cases of atypical BSE were confirmed. One of the two was diagnosed as atypical BSE at the age of 23 months (BSE/JP8), but the amount of PrP^{Sc} in the medulla oblongata was estimated to be 1/1,000 of that of classical BSE cattle and no infectivity of this medulla tissue was experimentally detected using Tg-mice. Therefore, FSCJ concludes that the infectivity to humans is negligible in this young atypical case.

Although the pathogenesis of atypical BSE is unknown, it is relevant to conduct an assessment regarding atypical BSE as a sporadic disease based on the reported prevalence situation.

7. Summary

1) The BSE status in cattle population in the five countries: Japan, the US, Canada, France and the Nether-

lands.

a) Japan

Since the first BSE case detected in 2001, 36 cases of BSE cattle in total have been diagnosed to date. No BSE cases have been confirmed among cattle born after tightening feed control regulation in October 2001, except one case born in January 2002. The results of surveillance show great effectiveness of the current feed regulation, although it is necessary to continue monitoring of infection/prevalence of BSE.

b) The US

Four cases of BSE have been confirmed so far. One of them was imported from Canada while the other three US natives were atypical BSE cases. As of September 2012, 35 months have passed since feed regulation was tightened in October 2009. Taking into account that the BSE incubation period is about 5 to 5.5 years, it is necessary to continue monitoring of infection/prevalence of BSE to ensure the efficacy of existing feed regulation. The US surveillance program for BSE cattle was aimed to detect one case in one million adult cattle, which is sufficient to meet a level of the OIE point system for detection of BSE at least one per 100,000 adult cattle.

c) Canada

So far 18 cases of BSE have been confirmed, excluding one imported from the UK. Since July 2007 when an existing feed regulation was tightened, no BSE case has been detected among cattle born in and after September 2004. As of September 2012, 62 months have passed since an existing feed regulation was tightened in July 2007. Taking into account that the BSE incubation period is about 5 to 5.5 years, it is necessary to continue monitoring of infection/prevalence of BSE for ensuring the efficacy of existing feed regulation. The BSE surveillance program in Canada was designed to detect at least one BSE positive cattle case at a confidence level in 95% where two per one million adult cattle are infected. The surveillance program meets the level of the OIE point system for detection of BSE at least one per 100,000 adult cattle.

d) France

So far 1,023 cases of BSE have been confirmed. Since a domestic feed regulation was tightened in November 2000, no BSE incidence by cohort has been confirmed except for two cattle cases born in 2001 and one cattle case born in April 2004. The results of surveillance show high effectiveness of the current feed regulation, although it is necessary to continue monitoring of infection/prevalence of BSE. The BSE surveillance level in France fulfills the BSE surveillance requirements implemented in EU legislation, thus meets the level of the OIE point system for detection of BSE at least one per 100,000 adult cattle.

e) The Netherlands

Eighty eight cases of BSE have been confirmed so far. Since a feed regulation was tightened in December 2000, no BSE incidence by cohort has been confirmed, except for one cattle case born in February 2001. The results of surveillance show high effectiveness of the current feed regulation, although it is necessary to continue monitoring of infection/prevalence of BSE. The BSE surveillance level in the Netherlands fulfills the BSE surveillance requirements implemented in EU legislation, thus meets the level of the OIE point system for detection of BSE at least one per 100,000 adult cattle.

2) Accumulation of PrP^{Sc} in bovine tissues and infection risks to humans

Based on the current BSE status in the five countries, as mentioned in Section 1 above, the amount of BSE prions taken by a head of cattle through feed, if any, may not exceed the level equivalent to 1 g of brain material of field BSE cases in the UK.

In the experiments exposing 1 g of the brain tissues of the UK BSE to cattle in the UK, clinical signs and PrP^{Sc} in the CNS were initially detected at 44 months post-exposure. PrP^{Sc} in the CNS was not detected in the cattle at 42 months post-exposure, i.e. over 46 months of age (M.E. Arnold *et al*. 2007; G.A.H. Wells *et al*. 2007). Furthermore, the results from the i.c. inoculation of BSE materials in cattle demonstrated that PrP^{Sc} in the brainstem was detected first at 7–8 months before onset of clinical signs. From these observations, the possibility is very low that the PrP^{Sc} could be detected in the CNS below the age of 30 months.

The incidence of vCJD peaked in 2000 in the UK, in which the highest number of cases have been reported, but then has gradually declined. Also vCJD cases in the world have markedly decreased from its peak to only several cases a year. It has been suggested that the incidence of vCJD is closely linked to that of BSE. FSCJ considers therefore that

BSE control measures such as the feed regulation and prohibition of the use of SRMs in food effectively reduced the risk of infection with the BSE agent not only in cattle but also in humans.

Infection risks of transmissible agents derived from atypical BSE cannot be ignored. Most of the atypical BSE cases were found in cattle aged over eight years (detection ages ranged from 6.3 to 18 years) excluding the one case in Japan that was found at the age of 23 months. The amount of abnormal prions was very small in medulla oblongata of this young atypical BSE case, so that no transmission was noted in the tissue even to highly susceptible Tg mice. Therefore, atypical BSE prions from cattle other than old one, if any, provoke negligible risks to human health.

3) Result of the assessment

FSCJ conducted risk assessments under the assumption that the present feed control measures are maintained in Japan, the US, Canada, France and the Netherlands. The assessments were also based on the current BSE status and infection risks in cattle in these countries, and interspecies barrier of BSE transmission between cattle and humans. Consequently, FSCJ concludes that in these countries, vCJD is highly unlikely to develop in association with consumption of BSE prions through meat and offal (excluding the tonsils and distal ileum) from cattle at or under 30 months of age.

FSCJ adopted therefore the following conclusions on the domestic measures and the border measures:

a) Domestic measures

a1. Age limits for BSE testing

Negligible influences to human health are predicted from the changes in the age limit for BSE testing of cattle from the current 20 months to 30 months.

a2. Age limits for SRMs in domestic cattle

Changes in definition on age of SRMs (skull excluding tonsils, spinal cord and vertebral column) from “all ages” to “over 30 months of age” have negligible influences to human health.

b) Border measures

b1. Age restriction on cattle meat and offal import

Negligible influences to human health are predicted from the changes in the age restriction from the current 20 months to 30 months on cattle meat and offal imported from the US and Canada. Negligible influences to human health are predicted between the current ban and implementation of the age restriction of 30 month on import of cattle meat and offal from France and the Netherlands.

b2. Age restriction for SRMs in cattle

Changes in definition on age of SRMs (skull excluding tonsils, spinal cord and vertebral column) from “all ages” (equivalent to “import ban” for France and the Netherland) have negligible influences to human health (**Table 17**).

Table 17. Status of BSE Confirmation Department of Food Safety, Pharmaceutical and Food Safety Bureau, MHLW (An electronic file of this table is available as a supplementary material.)

	Confirmation date (Date of slaughter/ death)	Birth date (Age in month at confirmation)	Variety (Sex)	Production place (Farm)	Authorities conducting inspection (Authorities implementing confirmation inspection)	Clinical symptoms, etc. (Note 2)	Results of confirmation inspection (Note 1)
1	10-Sep-01 (6 August 2001)	26-Mar-96 (64 months)	Holstein (Female)	Saroma-cho, Hokkaido (Shiroi-shi, Chiba-ken)	Chiba-ken (National Institute of Animal Health)	Astasia, sepsis	WB method + Immunohistochemical testing + Histopathologic testing +
2	21-Nov-01 (19 November 2001)	4-Apr-96 (67 months)	Holstein (Female)	Sarufutsu-mura, Hokkaido (Sarufutsu-mura, Hokkaido)	Ubushi Substation, Teshio Branch, Hokkaido Rumoi Health Center (Obihiro University of Agriculture and Veterinary Medicine)	None	WB method + Immunohistochemical testing + Histopathologic testing -
3	2-Dec-01 (29 November 2001)	26-Mar-96 (68 months)	Holstein (Female)	Miyagi-mura, Gunma-ken (Miyagi-mura, Gunma-ken)	Saitama Chuo Meat Inspection Center (Center for Inspection of Imported Foods and Infectious Diseases Yokohama Quarantine Station; Obihiro University of Agriculture and Veterinary Medicine)	None	WB method + Immunohistochemical testing + Histopathologic testing +
4	13-May-02 (10 May 2002)	23-Mar-96 (73 months)	Holstein (Female)	Onbetsu-cho, Hokkaido (Onbetsu-cho, Hokkaido)	Hokkaido Kushiro Health Center (Obihiro University of Agriculture and Veterinary Medicine)	Left front limb neuroparalysis, dysstasia	WB method + Immunohistochemical testing + Histopathologic testing +
5	23-Aug-02 (21 August 2002)	5-Dec-95 (80 months)	Holstein (Female)	Isehara-shi, Kanagawa-ken (Isehara-shi, Kanagawa-ken)	Meat Inspection Station, Kanagawa Prefectural Government (National Institute of Infectious Diseases)	Astasia, hip dislocation, arthritis in both front limbs, garget, heat stroke	WB method + Immunohistochemical testing + Histopathologic testing -
6	20-Jan-03 (17 January 2003)	10-Feb-96 (83 months)	Holstein (Female)	Shibecha-cho, Hokkaido (Kokawa-cho, Wakayama-ken)	Meat Inspection Office, Wakayama City Public Health Center (National Institute of Infectious Diseases)	Dysstasia	WB method + Immunohistochemical testing + Histopathologic testing +

Table 17. (continued.)

7	23-Jan-03 (21 January 2003)	28-Mar-96 (81months)	Holstein (Female)	Yubetsu-cho, Hokkaido (Abashiri-shi, Hokkaido)	Hokkaido Kitami Health Center (Obihiro University of Agriculture and Veterinary Medicine)	None	WB method + Immunohistochemical testing + Histopathologic testing -
8	6-Oct-03 (29 September 2003)	13-Oct-01 (23 months)	Holstein (Castrated)	Ohtawara-shi, Tochigi-ken (Katsurao-mura, Futaba-gun, Fukushima-ken)	Kenhoku Meat Inspection Center (Ibaraki Prefecture) (National Institute of Infectious Diseases)	None	WB method + (Note 3) Immunohistochemical testing - Histopathologic testing -
9	4-Nov-03 (29 October 2003)	13-Jan-02 (21 months)	Holstein (Castrated)	Hikami-gun, Hyogo-ken (Fukuyama-shi, Hiroshima-ken)	Fukuyama City Meat Sanitation Inspection Station (National Institute of Infectious Diseases)	None	WB method + Immunohistochemical testing - Histopathologic testing -
10	22-Feb-04 (20 February 2004)	17-Mar-96 (95 months)	Holstein (Female)	Hadano-shi, Kanagawa-ken (Hiratsuka-shi, Kanagawa-ken)	Meat Inspection Station, Kanagawa Prefectural Government (National Institute of Infectious Diseases)	Dysstasia, hit dislocation	WB method + Immunohistochemical testing + Histopathologic testing +
11 (Note 4)	9-Mar-04 (4 March 2004)	8-Apr-96 (94 months)	Holstein (Female)	Shibecha-cho, Hokkaido (Shibecha-cho, Hokkaido)	Tokachi Animal Health Center (National Institute of Animal Health)	Hip dislocation (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing +
12	13-Sep-04 (10 September 2004)	3-Jul-99 (62 months)	Holstein (Female)	Shisui-machi, Kumamoto-ken (Shisui-machi, Kumamoto-ken)	Kumamoto Prefectural Meat Inspection Office (National Institute of Infectious Diseases)	None	WB method + Immunohistochemical testing + Histopathologic testing +
13	23-Sep-04 (21 September 2004)	18-Feb-96 (103 months)	Holstein (Female)	Shihoro-cho, Hokkaido (Shinjo-cho, Nara-ken)	National Institute of Health Sciences, Nara Prefecture (National Institute of Infectious Diseases)	Astasia, hip dislocation	WB method + Immunohistochemical testing + Histopathologic testing +

Table 17. (continued.)

14	14-Oct-04	8-Oct-00	Holstein	Shikaoi-cho, Hokkaido	Tokachi Animal Health Center	Death from suffocation	WB method +
(Note 4)	(8 October 2004)	(48 months)	(Female)	(Shikaoi-cho, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing +
15	26-Feb-05	5-Aug-96	Holstein	Honbetsu-cho, Nakagawa-gun, Hokkaido	Tokachi Animal Health Center	Arthritis	WB method +
(Note 4)	(22 February 2005)	(102 months)	(Female)	(Honbetsu-cho, Nakagawa-gun, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing +
16	27-Mar-05	23-Mar-96	Holstein	Teshio-cho, Hokkaido	Asahikawa City Meat Hygiene Inspection Center	None	WB method +
	(24 March 2005)	(108 months)	(Female)	(Teshio-cho Hokkaido)	(National Institute of Infectious Diseases, Obihiro University of Agriculture and Veterinary Medicine)		Immunohistochemical testing +
							Histopathologic testing +
17	8-Apr-05	11-Sep-00	Holstein	Otofuke-cho, Kato-gun, Hokkaido	Tokachi Animal Health Center	Astasia	WB method +
(Note 4)	(4 April 2005)	(54 months)	(Female)	(Otofuke-cho, Kato-gun, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing +
18	12-May-05	31-Aug-99	Holstein	Sunagawa-shi, Hokkaido	Hokkaido Hayakita Meat Inspection Center	Astasia, dislocation of both hips	WB method +
	(10 May 2005)	(68 months)	(Female)	(Sunagawa-shi, Hokkaido)	(Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)		Immunohistochemical testing +
							Histopathologic testing +
19	2-Jun-05	16-Apr-96	Holstein	Betsukai-cho, Notsuke-gun, Hokkaido	Health and Welfare Department, Health and Welfare Office of Kushiro, Hokkaido	None	WB method +
	(31 May 2005)	(109 months)	(Female)	(Betsukai-cho, Notsuke-gun, Hokkaido)	(Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)		Immunohistochemical testing +
							Histopathologic testing -

Table 17. (continued.)

20	6-Jun-05 (3 June 2005)	12-Aug-00 (57 months)	Holstein (Female)	Shikaoi-cho, Kato-gun, Hokkaido (Shikaoi-cho, Kato-gun, Hokkaido)	Hokkaido Obihiro Meat Inspection Center (Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)	None	WB method + Immunohistochemical testing + Histopathologic testing -
21 (Note 4)	10-Dec-05 (6 December 2005)	13-Feb-00 (69 months)	Holstein (Female)	Chitose-shi, Hokkaido (Chitose-shi, Hokkaido)	Ishikari Livestock Hygiene Service Center (National Institute of Animal Health)	Cardiac insufficiency (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing -
22 (Note 4)	23-Jan-06 (20 January 2006)	1-Sep-00 (64 months)	Holstein (Female)	Betsukai-cho, Notsuke-gun, Hokkaido (Betsukai-cho, Notsuke-gun, Hokkaido)	Nemuro Livestock Hygiene Service Center (National Institute of Animal Health)	Variation of left abomasums (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing Undeterminable (Note 5)
23	15-Mar-06 (13 March 2006)	8-Jul-00 (68 months)	Holstein (Female)	Nakagawa-cho, Nakagawa-gun, Hokkaido (Nakagawa-cho, Nakagawa-gun, Hokkaido)	Health Department of Nayoro Region, Health and Welfare Office of Kamikawa, Hokkaido (Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)	None	WB method + Immunohistochemical testing + Histopathologic testing +
24	17-Mar-06 (13 March 2006)	10-Feb-92 (169 months)	Japanese Black Cattle	Iki-shi, Nagasaki-ken (Iki-shi, Nagasaki-ken)	Sasebo Meat Inspection Center (National Institute of Infectious Diseases)	Astasia	WB method + (Note 6) Immunohistochemical testing + Histopathologic testing +
25	19-Apr-06 (17 April 2006)	18-Apr-00 (71 months)	Holstein (Female)	Esashi-cho, Esashi-gun, Hokkaido (Nagi-cho, Okayama-ken)	Meat Inspection Center, Okayama Prefectural Government (National Institute of Infectious Diseases)	None	WB method + Immunohistochemical testing + Histopathologic testing -
26 (Note 4)	13-May-06 (10 May 2006)	11-Aug-00 (68 months)	Holstein (Female)	Imakane-cho, Setana-gun, Hokkaido (Imakane-cho, Setana-gun, Hokkaido)	Ishikari Livestock Hygiene Service Center (National Institute of Animal Health)	Arthritis (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing Undeterminable (Note 5)

Table 17. (continued.)

27 (Note 4)	19-May-06 (16 May 2006)	20-Aug-00 (68 months)	Holstein (Female)	Toyokoro-cho, Nakagawa-gun, Hokkaido (Toyokoro-cho, Nakagawa-gun, Hokkaido)	Tokachi Animal Health Center (National Institute of Animal Health)	Garget (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing +
28 (Note 4)	11-Aug-06 (7 August 2006)	21-Nov-99 (80 months)	Holstein (Female)	Horonobe-cho, Teshio-gun, Hokkaido (Haboro-cho, Tomamae-gun, Hokkaido)	Ishikari Livestock Hygiene Service Center (National Institute of Animal Health)	Debility cordis, dislocation of the right hip (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing -
29 (Note 4)	28-Sep-06 (24 September 2006)	24-Jun-00 (75 months)	Holstein (Female)	Horonobe-cho, Teshio-gun, Hokkaido (Nakagawa-cho, Nakagawa-gun, Hokkaido)	Ishikari Livestock Hygiene Service Center (National Institute of Animal Health)	Ketosis (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing +
30 (Note 4)	13-Nov-06 (8 November 2006)	28-Jun-01 (64 months)	Holstein (Female)	Chitose-shi, Hokkaido (Chitose-shi, Hokkaido)	Ishikari Livestock Hygiene Service Center (National Institute of Animal Health)	Cardiac insufficiency (Dead cow)	WB method + Immunohistochemical testing + Histopathologic testing Undeterminable (Note 5)
31	8-Dec-06 (6 December 2006)	12-Nov-99 (84 months)	Holstein (Female)	Shikaoi-cho, Kato-gun, Hokkaido (Shikaoi-cho, Kato-gun, Hokkaido)	Hokkaido Obihiro Meat Inspection Center (Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)	Tachypnea, gait staggering	WB method + Immunohistochemical testing + Histopathologic testing -
32	5-Feb-07 (2 February 2007)	26-Aug-01 (65 months)	Holstein (Female)	Obihiro-shi, Hokkaido (Obihiro-shi, Hokkaido)	Hokkaido Obihiro Meat Inspection Center (Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)	Left hip swelling	WB method + Immunohistochemical testing + Histopathologic testing +

Table 17. (continued.)

	2-Jul-07	21-Jun-00	Japanese Black Cattle	Makubetsu-cho, Nakagawa-gun, Hokkaido	Tokachi Animal Health Center	Fatty liver	WB method +
33 (Note 4)	(24 June 2007)	(84 months)		(Makubetsu-cho, Nakagawa-gun, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing Undeterminable (Note 5)
	21-Dec-07	1-Jul-92	Japanese Black Cattle	Shimane-ken	Hokkaido Yakumo Meat Inspection Center	None	WB method +
34	(19 December 2007)	(185 months)		(Niikappu-cho, Niikappu-gun, Hokkaido; Setana-cho, Kudo-gun, Hokkaido)	(Hokkaido University, Obihiro University of Agriculture and Veterinary Medicine)		Immunohistochemical testing +
							Histopathologic testing -
	24-Mar-08	12-Oct-00	Japanese Black Cattle	Biratori-cho, Saru-gun, Hokkaido	Ishikari Livestock Hygiene Service Center	Cardiac insufficiency	WB method +
35 (Note 4)	(17 March 2008)	(89 months)		(Rumoi-shi, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing Undeterminable (Note 5)
	30-Jan-09	5-Aug-00	Holstein	Imakane-cho, Setana-gun, Hokkaido	Ishikari Livestock Hygiene Service Center	Dysstasia	WB method +
36 (Note 4)	(26 January 2009)	(101 months)	(Female)	(Imakane-cho, Setana-gun, Hokkaido)	(National Institute of Animal Health)	(Dead cow)	Immunohistochemical testing +
							Histopathologic testing +

(Note 1) A result of histopathologic testing is indicated as "+" if vacuoles were clearly confirmed in brain tissues.

(Note 2) In either case, no BSE-suspicious clinical symptom was confirmed.

(Note 3) Sugar chain pattern and protease resistance were different from those of BSE confirmed so far.

(Note 4) This cow was confirmed during an examination of dead cattle in the production process and was not transported to a slaughterhouse.

(Note 5) Although vacuolar degeneration was confirmed, the result is "undeterminable" on ground that a clear distinction from postmortem change was difficult.

(Note 6) The pattern of PrP^{Sc} detected was not typical.

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