Tentative Translation

Measures against Bovine Spongiform Encephalopathy (BSE) in Japan

(Interim report)

September 2004

Food Safety Commission

Contents

1. Introduction

2. Background

2-1. BSE

2-1-1.The number of BSE-cattle

2-1-2. Incubation period of BSE

2-1-3. In vivo prion distribution and infectivity

2-1-4. Mechanisms of BSE development

2-2. vCJD

2-2-1.The number of vCJD patients

2-2-2. Incubation period of vCJD and minimum amount required for vCJD development

2-2-3. Interspecies barrier between cattle and humans

2-2-4. Genetic factors involved in vCJD infection

3. Risk evaluation

3-1. Basic principles for risk evaluation

3-2. A case of risk evaluation in the UK (Estimation of the number of infected patients and prediction of vCJD occurrence)

3-3. Risk evaluation in Japan

3-3-1. Estimation of the number of vCJD occurrence by risk evaluation of the past

3-3-1-1.The number of BSE-cattle involved in the human food chain and predicted number of BSE-cattle in the future.

3-3-1-2. Evaluation of vCJD risk in Japan based on the estimation of vCJD patients in the UK simply on a pro-rata basis.

3-3-2. Risk reduction through control measures

3-3-2-1. Countermeasures against BSE occurrence

- Management and regulation of animal feed
- Implementation of traceability program
- Testing risk cattle

3-3-2-2. Risk reduction through BSE test, its detection limit and significance

- Detection limit of BSE prions by rapid test
- Detectable age by rapid test

・ Prospects of test

3-3-2-3. Risk reduction by SRM (specified risk materials) removal

・ SRM removal

・ Contamination during slaughtering

3-3-3. Current risks

3-3-4. Increase and decrease of the risk in accordance with the selection of management measures

4. Conclusions

5. Closing remarks

(Abbreviations)

(References)

1. Introduction

In Japan, September 10th 2001, identification of suspected Bovine Spongiform Encephalopathy (BSE) cows was reported. This news stunned the people involved in the livestock industry. In addition, an effective cure is not yet available for variant Creutzfeldt - Jakob disease (vCJD), which is supposedly caused by transmission of the BSE agent (BSE prion) to humans, thus all infected people will succumb to the disease. These facts and others threw the whole nation into a panic. In response to the situation, on October 18th, a month after the detection, the Ministry of Health, Labour and Welfare and the Ministry of Agriculture, Forestry and Fisheries of Japan have implemented a range of measures including testing all the cattle and removal of specified risk materials (SRM), which were deemed stricter than those implemented in European countries.

In 1986, BSE was identified in the UK and, in 1988, reported as a new disease by the British Government at the 56th annual general meeting of Office International des Epizooties (OIE). After that, almost all the BSE-cases in the 1990s were discovered in the UK. Since 2001, BSE tests have started in slaughterhouses in the EU by using a rapid diagnostic method introduced at the end of the 1990s. Following this, the number of countries, where additional BSE-cattle were discovered, has increased to 23 countries worldwide but mainly European countries (the United States was excluded from the statistics, so one case discovered there was added to the counting of Canada) and more than 180,000 cattle infected with BSE prion (hereinafter called BSE-cattle) have been confirmed. According to the epidemiological investigation performed in the UK, the cause of the global spreading of BSE was due to Meat and Bone Meal (MBM) derived from BSE-cattle that was used to feed cows.

In Japan, three years have passed since the first discovery of BSE-cattle. Results of testing of all the beef cows discovered 9 BSE-cattle, moreover, fully implemented tests of all the dead cattle at 24-month-old and above, which was initiated in April 2004, discovered one additional BSE-cow. These results have given us a rough understanding of the situation of BSE infection over a short duration. Although limited research achievements on

the BSE mechanisms have been obtained, the mechanisms of the vCJD development after a BSE infection remained unsolved. As described above, scientific findings obtained so far are limited, however this report was finalized based on these findings to review the effects of decreasing the risks of cattle-to-human BSE infection and the measures against BSE (management and measures) in Japan and to utilize the findings for measures in the future.

BSE-related risks are relevant to transmissions as follows: ① cow-to-cow, ② cow-to-human, ③ human-to-human. With recognition of these three viewpoints, the committee has reviewed the risk of ② cow-to-human transmission of BSE prions under ordinary eating habits.

2. Background

2-1. BSE

2-1-1.The number of BSE-cattle

OIE reports say that there are 188,760 BSE-cattle in 23 countries across the globe (as of July 22nd 2004), the number by country is 183,880 in the UK, which makes up the vast majority, and the rest are as follows: in Ireland (1426 heads), in France (914 heads), in Portugal (904 heads), in Switzerland (454 heads) (Table.1)

Meanwhile, in Japan, 11 BSE-cattle have been confirmed up to now. In September 2001, discovery of the first case of BSE-cattle was the trigger for testing all the cattle in slaughterhouses on October 18th in the same year. After testing 3,451,152 cattle, 9 BSE-cattle have been confirmed until now. (Statistics of the Ministry of Health, Labour and Welfare; as of July 31st 2004) (Table.2) So far, surveillance of dead cattle has tested 69,218 cattle, although the number is insufficient, and one of them was diagnosed as a BSE-cow. (Statistics of the Ministry of Agriculture, Forestry and Fisheries of Japan; between October 18th 2001 and May 31st 2004) (Table.3)

In 11 BSE-cattle cases confirmed in Japan, no typical clinical signs that are an index of suspicion of BSE infection were observed, but 6 cattle showed some clinical signs such as dysstasia and sepsis. (Table.4)

Birthplaces of infected cattle by prefecture are as follows: 6 cases out of 11 are in Hokkaido, 2 cases in Kanagawa, one case in Gunma, Tochigi, and Hyogo, respectively.

Breakdown by the period of birth is as follows: 9 cases concentrate on the

period between December 1995 and April 1996; 2 young cases are between October 2001 and January 2002. (Table.4, Fig. 1) Grouping by category is as follows: 2 young cases are male dairy breed (Holstein-Friesian castrated）; the other 9 cases are female dairy breed (Holstein-Friesian).

Among these, the 8th case of BSE-cattle (23-month old) was diagnosed as negative by immunohistochemical and histopathological diagnosis, however, a positive reading was obtained by Western Blotting (hereinafter called “WB method”). Considering the low resistance to proteinase and the different pattern of electrophoresis from those observed in abnormal prion protein by the WB method, this case was diagnosed as being “atypical BSE” by “The Prion Expert Committee for testing bovine spongiform encephalopathy (BSE)” held by the Ministry of Health, Labour and Welfare.

In addition, the 9th case of BSE-cattle (21-month old) was diagnosed as negative by immunohistochemical and histopathological diagnosis, but as positive by the WB method as with the 8th case and showed the same characteristics of those observed in up to the 7th case of BSE-cattle by the WB method.

With respect to the 8th and 9th cases from the result of the WB method, in comparison with the other 9 cases, the amount of abnormal prion protein accumulated in the medulla oblongata is small, which is estimated at 1/500th to 1/1,000th less than those in the other cases[1)]. In order to characterize the BSE prions of these 2 cases, brain homogenates were inoculated into bovine transgenic mice, and experiments are now being continued to determine the amplification of the BSE prions, whose results are expected to reveal the transmissibility of the 2 cases.

For additional up-to-date information regarding the number of BSE-cattle worldwide, visit the OIE site (http://www.oie.int/eng/info/en_esb.htm)

2-1-2. Incubation period of BSE

The incubation period of BSE is presumed to be an average of 5 years (60 months) and 4~6 years (48~72 months) in most cases, based on the data of natural BSE-cattle observed in the UK. However, the incubation period is believed to vary with individual differences or amount of exposure.

In the UK, the youngest reported and the oldest BSE-case were at 20-month-old and 19-year-old, respectively[2)].

And concerning the ages of the 11 BSE-cattle confirmed in Japan at the time

of slaughter, 2 young cases were at 21 to 23-month-old and the mean age of the other 9 cases was at 78.3±10.7-month-old, and none of them showed typical BSE-symptoms. (Table.4)

2-1-3.In vivo prion distribution and infectivity

The BSE prion amplifies itself over the long term in the living bodies of infected cattle. Infectivity of a cow depends on the number of years after infection and on the passage of years during the incubation period, but the temporal profile of the prion accumulation is unclear for all practical purposes. We can only guess as to the period of infection, even if we follow the preexisting presumptions that BSE-cattle were infected soon after birth. In this regard, the accumulation level of abnormal prion protein in younger BSE-cattle is presumed to be much lower than that in BSE-cattle at the last stage of the incubation period[3].

In respect to BSE prion distribution in the living bodies of BSE-cattle, data of infection experiments performed by the UK Central Veterinary Laboratory are available[4]. In this experiment, 40 calves at 4-month-old were gathered from the farms which had no history of BSE infection, and 100g of brain tissue collected from 75 BSE-cattle was orally administered to 30 calves, the other 10 calves, used as a control group, were not administered brain tissue. Between 6 and 22 months after administration, every 4 months, 3 calves from the administered group and one control calf were slaughtered. After the period, up to 40 months after administration, calves were slaughtered if necessary. The infectivity of the collected tissues was tested by inoculation into the brain and abdominal cavity of mice (mouse bioassay) and by inoculation into the brain of cattle (cattle bioassay). In the mouse bioassay, 44 tissues were collected (mainly lymphoreticular system, peripheral nervous system, central nervous system, gastrointestinal tract, striated muscle, and major organs) the tissue samples were suspended in saline to prepare 10% solution. This solution was administered into the brain (amount of inoculation: 20μl) or abdominal cavity (amount of inoculation: 100μl) of inbred mice. In the cattle bioassay, several tissues were selected based upon the results of the mouse bioassay.

The results revealed infectivity of the cattle brain (50% infectivity after inoculating into the brain: $\leqq 10^{3.2} \sim 10^{5.6}$C.i.c.$ID_{50}$/g: ID_{50} indicates the amount of inoculum required for the establishment of infection or BSE development of 50% of

cattle) and of the spinal cord, dorsal root ganglion, trigeminal ganglion ($\leqq 10^{3.2}$C.i.c.ID_{50}/g) removed at 32~40 months after administration, and distal part of ileum ($\leqq 10^{3.3}$〜$10^{5.6}$C.i.c.ID_{50}/g) at 6~18 months after administration. No infectivity, however, was confirmed in all the tested tissues at 22 or 26 months after administration. (Table.5) In addition, only one case showed extremely low levels of infectivity in the bone marrow (breast bone) at 38 months after administration revealed by the mouse bioassay[5]. Moreover, the cattle bioassay showed infectivity in the tonsils at 10 months after administration[6]. Clinical signs were observed after 35 months. However, only a small number of cattle were used for the test, and the results obtained were based on the observations of one or a few cattle. Moreover, the inspection method cannot detect the infectivity below its detection threshold. Considering these facts, there remained uncertainty that the possibility of the presence of infection under the detection threshold cannot be excluded, even if infection could not be confirmed in certain tissue[3].

At the medulla oblongata (obex), the spinal tract nucleus of the trigeminal ganglion, the nuclei of solitary tract and the dorsal nucleus of the vagus nerve are gathered, and it's noted that spongy degeneration is frequently observed in the neuropile and nerve cells in these tissues of BSE-cattle[7),8)]. Therefore, the medulla oblongata (obex) carries the largest amount of abnormal prion proteins among all the tissues with infectivity confirmed[9)].

In addition, from the results of the infection experiment performed by the UK Central Veterinary Laboratory, the total infective dose of a cow which developed BSE symptoms was estimated to be approximately 8,000 infective dose cattle oral ID_{50s} (C.o.ID_{50}), and it is estimated that SRM accounts for more than 99% of the infective dose[3]. (Table 6) However, this is an estimation extrapolated with results obtained by testing scrapie, sheep prion disease.

Currently, experiments are in progress as follows: At the UK Central Veterinary Laboratory, oral administration of 100g or 1g of BSE brain tissue to 100 cattle each, and also in Germany, oral administration of BSE brain tissue to 56 cattle. In Japan, experiments of the same kind have been started. These experiments or more sensitive diagnostic methods, if developed, are expected to produce results to yield new findings.

In addition, in regard to the minimum amount of BSE prions (threshold dose) required to develop BSE, experiments of oral administration of

smaller amounts of BSE brain tissue (0.1, 0.01, and 0.001g) are now in progress at the UK Central Veterinary Laboratory. Results obtained to date are as follows: 3 out of 15 cattle in the 0.1g administration group, 1 out of 15 cattle in the 0.01g administration group, 1 out of 15 cattle in the 0.001 administration group developed BSE. Since experiments of oral administration using smaller amounts of brain tissue were not performed, however, the threshold dose cannot be determined by this study.

2-1-4. Mechanisms of BSE development

Mechanisms of BSE development in cattle are unclear. Experiments on the scrapie development using sheep or rodents by oral administration of scrapie prions revealed the accumulation of scrapie prions at the Peyer patches of the ileum, lymphatic tissues attached to the intestine, and at the intestinal visceral nerves distributed through the intestine. These results lead to the hypothesis that scrapie prions spread to the central nervous system via intestinal, visceral, or vagus nerve[10]. It remains unclear how the scrapie prion reaches the peripheral nerve terminal from the follicular dendritic cells.

In fact, it takes a prolonged period for the BSE prions to accumulate in the central nervous system and results in spongy degeneration in the brain followed by the development of BSE symptoms. Thus, any test using samples extracted from the medulla oblongata (obex) can detect BSE-cattle only in the latter stage of the incubation period. Whether other organs are infective or not is unclear at present. At the Federal Institute for Risk Assessment (BfR German), in vivo pathological studies are being continued regarding the temporal and spatial transmission profiles of BSE prion after the infection[11].

As observed above, a range of basic studies are in progress in regards to the in vivo manners of transmission, distribution, and amplification, but large parts of them remain to be answered. Further support and progress in the future are being awaited.

2-2. vCJD

2-2-1.The number of vCJD patients

There are a total of 157 vCJD cases worldwide. Among them, 147 cases were found in the UK, and have the same tendency as observed in BSE-cases.

Other vCJD cases were confirmed in other countries as follows: {France (6 cases), Italy (1 case), Ireland (1 case), Canada (1 case), and the U.S.A. (1 case)} All the patients except 5 French patients and 1 Italian patient have a history of stay in the UK. (Table1)
In the meantime, not a solitary patient has been reported in Japan. (as of the end of June 2004)

2-2-2.Incubation period of vCJD and minimum amount required for vCJD development

The mechanisms for the BSE prions to spread to the central nervous system after infection in humans and its temporal progression are unknown[12].

Also, the length of the incubation period is unclear; it ranges from a few to more than 25 years according to one hypothesis[3]. Although no patients have been found in Japan so far, this does not necessarily mean that patients will not be identified in the future[3].
Furthermore, the relationship between the infective dose and vCJD development in humans, especially the minimum amount for vCJD development and cumulative effects by repeated administration are still unknown.

2-2-3.Interspecies barrier between cattle and humans

The degree of an interspecies barrier in the transmission of BSE prions from cattle to humans is under consideration by the Task Force of the Science Steering Committee (SSC) in the European Commission. According to the report[12], evaluation of BSE prions, vCJD prions, and scrapie prions by the experiments using different animal species, specially developed transgenic mice, and cultured cells have led to the assumption that an interspecies barrier between cattle and humans exists. However, based on current knowledge, quantification of its strength is deemed impossible.
Based on the prior acknowledgement, the Task Force considers it desirable to assume a worst-case scenario; that the barrier does not exist until new scientific data concerning the interspecies barrier is available to evaluate the risks of human exposure to potentially BSE-contaminated products. Meanwhile, the interspecies barrier is estimated to be 10~10,000 times higher, if the range of the minimum infectious dose estimated among a

variety of animal species is applied to humans. But, in parallel, recommendations have been made to develop an appropriate model based on the interspecies barrier for risk assessment on an international collaboration basis.
Therefore, the following 2 approaches are considered possible: ① Quantitative evaluation of BSE prions required to cause vCJD in humans by crossing the interspecies barrier between cattle and humans ② Evaluation of vCJD risks in Japan based on epidemiological data such as correlation between the numbers of BSE cases and vCJD cases in UK. The risk assessment by approach① is deemed extremely difficult because of the wide variety of estimated values concerning the degree of interspecies barrier between cattle and humans, and the lack of an appropriate international assessment model.

2-2-4.Genetic factors involved in vCJD infection

All vCJD patients reported to date in the UK, have a gene encoding prion protein with codon129 methionine (methionine/methionine; M/M) homozygotes, except for the 2nd case infected via blood transfusion. It has been pointed out that vCJD patients with this genotype present a shorter incubation period of vCJD, more susceptibility to infection than other genotypes or either one of them[3].
It is reported that approximately 40% of Caucasians in Europe including the UK share M/M homozyotes, 13% of Valine homozyotes (Valine/Valine; V/V), and 47% share heterozygous genotype (Methionine/Valine; MV) [13,14].
In this regard, in Japan, the ratio of the population with M/M genotype is assumed to be higher than the UK[15,16], for example 91.6% in one report[17].
In the UK, a second case of predicted human-to-human transmission of vCJD through blood transfusion was confirmed. This patient succumbed to a non-related disease without any vCJD symptoms, but the following examination result was found upon confirming his infection. The genotype was reported as M/V in this case, which differs from those of other vCJD patients[18].

3. Risk evaluation

3-1. Basic principles for risk evaluation

In this document, measures against BSE in Japan will be evaluated based

on the following principles:
(1) Measures against BSE which transmit from cattle to humans should be evaluated by the human risk of BSE infection in Japan as an indicator, which were made based on the estimations of risk determined in the UK, considering the lack of any appropriate international models.
(2) Human risks of BSE infection in Japan will be evaluated before and after implementing measures against BSE of the past, or in case alterations are made in the future.
(3) Measures for risk management implemented in Japan are as follows: testing all the cattle in slaughterhouses, SRM removal, improving the methods of slaughtering and processing, measurements and regulations of feed, testing dead cattle, and traceability. While monitoring the implementation of these measures, effects of these measures will be evaluated.
(4) Findings obtained to date, for example diagnostic data of BSE, will be analyzed and assembled for the evaluation of human risks of BSE infection in Japan.
(5) With respect to BSE itself, risk assessment will be performed considering the limits of scientific and biological findings and a large number of issues which remain scientifically uncertain.
(6) Specific measures for risk management should be laid down by risk-management ministries based on these risk assessments and deep mutual understanding of BSE-risks through intensive risk communications.

3-2. A case of risk evaluation in the UK (Estimation of the number of infected patients and prediction of vCJD occurrence)

As has been mentioned previously the greatest number of BSE-cattle have been identified in the UK. Although 147 cases of human vCJD have been reported to date, no prediction can be made concerning the number of infected patients in the future. In the UK, examination of abnormal prion proteins was performed in 12,647 samples of appendices removed from patients who received appendectomies, and 3 samples turned out positive. When this number is applied to the population of the UK, the total number of infected patients in the incubation period are estimated to be up to approximately 3,800[19]. Furthermore, in December 2003 and July 2004, potential vCJD-cases infected via blood transfusion were reported[18, 20].

In the UK, P. Smith (chairman of Spongiform Encephalopathy Advisory Committee and a professor of London University) et al proposed a hypothesis which can explain the relationship between the numbers of BSE-cattle of the past and the occurrences of vCJD patients to date, and they predicted the number of occurrences of vCJD patients according to this hypothesis[21].

This prediction is based on a number of assumptions as follows: ① The number of vCJD-patients correlates with that of BSE-cattle at the time when measures were inadequate. ② Considerable individual variation can exist with respect to the incubation period, but it follows a specific statistical distribution. ③ No correlation is assumed between the incubation period and the ages of infection. ④ vCJD only occurs among those whose genotype is M/M at amino-acid residue 129 in the prion protein gene. ⑤ Patients during the incubation period are excluded. ⑥ The relationship between the quantity of prions and the incidence rate is not taken into consideration. Following this prediction, the total sum of vCJD patients is estimated from a few hundred to a few thousand.

In addition, in the UK, cows at 30-month-old or older are not used for food, but in case this regulation is lifted, Smith et al proposed a prediction concerning the increase of risks. Their most pessimistic estimation is based on the prediction that the total sum of patients would be 5,000.

Other predictions have also been reported regarding the number of vCJD patients in the UK. Thomas and Newby estimated numbers less than several hundred based on the clinical data of 23 patients who died between 1995 and 1997. Moreover, J.N. Huillard et al. estimated a number of several thousands at most based on the data obtained from 82 vCJD patients who developed symptoms before 2000, but they concluded that the number of infected people was unpredictable[23].

Meanwhile, since the genotype of the suspected case after blood transfusion found in 2004 was M/V, the past predictions of vCJD occurrences need reviewing[18].

3-3. Risk evaluation in Japan

As mentioned above, in order to evaluate the risk of vCJD in Japan, the following methods are possible: ① Risk evaluation of each step toward human consumption of BSE prions: How much BSE prion is contaminated

in the food chain? How much of it will cross the interspecies barrier? How many people will be at risk of vCJD? Based on the risk evaluation at each step toward human consumption of BSE prions, conclusive evaluation of the risk will be made. ② Epidemiological method: Based on the hypothesis in the previous section proposed by P. Smith that the number of vCJD patients correlate with that of BSE-cattle in the UK, epidemiological data including the number of BSE-cattle of the past and the number of vCJD -patients reported to date can be applied to predict the number of vCJD patients in the future.

However, with respect to method ①, implementation is difficult for the following reasons: There is a wide variability of values obtained regarding the interspecies barrier as described previously. Causative food products involved in consumption of BSE prions in the UK and in Japan are not known. Causative differences in eating habits between these two nations are unclear, the minimum quantity required for vCJD development in human is unknown, and cumulative effects are unclear.

Thus, implementation of an epidemiological method is possible if several assumptions are made, and examples of its estimation are described below.

3-3-1. Estimation of the number of vCJD occurrence by risk evaluation of the past

3-3-1-1. The number of BSE-cattle involved in the human food chain and predicted occurrence of BSE-cattle in the future.

Assume that the progression of BSE prion accumulation in living bodies of cattle will follow a regular process.

(Estimation 1)

Earlier than October 2001, before the implementation of measures against BSE, vCJD risks derived from the exposure to BSE prions can be traced to those derived from contamination of BSE-cattle into the food chain caused by the lack of SRM removal and BSE test. Based on the estimation obtained by applying the age distribution of BSE-cattle detected in EU countries in 2001 to the month-ages of BSE-cattle in Japan at the time they were slaughtered, these BSE-cattle consisted of up to 5 cows whose estimated slaughter ages were as follows: three 5-year-old BSE-cattle and two 4-year-old BSE-cattle at the time they were slaughtered by October 2001[24].

In addition, according to the prediction in the report published in September 2003 by the Epidemiological Surveillance team of the Ministry of Agriculture, Forestry and Fisheries of Japan, the number of BSE-cattle in the future is estimated to be less than 30; the causes of infections are imported live cattle, cattle MBM, and animal fat [25]. Moreover, taking into account the rendering of an additional four BSE-cattle including young two cows (21-, 23-month-old) found after the publication of the report of the Ministry of Agriculture, Forestry and Fisheries of Japan, there is a possibility that up to 60 BSE-cattle will be confirmed after 2005-6 [26].

(Estimation 2)

The vCJD risk in Japan is derived from the contamination of BSE prions into the food chain. Estimation of BSE-cattle contaminate in the human food chain was based on the hypotheses as follows: 1) According to the birth periods of 11 BSE-cattle confirmed to date, BSE caused by exposure to BSE prions occurred in the following cohorts（group of herd with the same period of birth）: ①1995-96 ②2001-02. (Table.4, Fig. 1) 2). A worst-case scenario assumed that the number of detected BSE-cattle in the future would correlate with that of slaughtered cattle of every age on the grounds that all the cattle in both cohorts established by period of birth were contaminated at the same rate. The grounds for concluding that the detected BSE-cattle belonged to the cohorts described above were that BSE-cattle should have been discovered at higher rates at this time, after a lapse of 8 years, if BSE risks were higher in the cohorts before 1995~96. Moreover, after October 2001, the use of MBM as feed that derived from livestock as well as cattle was prohibited and BSE tests of all cattle and SRM removal were implemented, BSE prions were considered to be excluded from the human food chain. As a result, vCJD risks were presumed to become a thing of the past. Therefore, vCJD risks in Japan derive from the contamination of undetected BSE-cattle into the feed chain, which belong to the cohort of 1995~96 birth period and were slaughtered before October 2001.

① Steers which belong to 1995~96 birth period cohort

Considering the usual slaughtering ages of steers (Table.7, Fig.2), a large percentage of them were considered to have been slaughtered within 3 years

(1996~99). In the meantime, how many BSE-cattle were contaminated into the human food chain is unclear.

② Cows which belong to 1995~96 birth period cohort

9 BSE-cattle have been confirmed. The breakdown is as follows: three 5-year-old cows December 2000 ~March 2002, four 6-year-old cows December 2001 ~March 2003, two 7-year-old cows December 2002 ~March 2004. Based on these facts, BSE infection rates can be calculated from this cohort. The estimated number of BSE-cattle is obtained as follows: Multiply the BSE infection rate by the number of slaughtered cattle for every slaughtering age.

{Estimated number of BSE-cattle} = {BSE infection rate of the cohort} × {The number of slaughtered cattle in one year}・・・(①)

$$\text{In this regard, } \{\text{BSE infection rate of the cohort}\} = \frac{A}{B}$$

{A = 3+4+2 ＝ 9：The number of BSE-cattle at 5~7-year-old}

{B =30,391+28,994+24,219=83,604：The number of slaughtered cattle at 5~7-year-old}

- Dec.1995~Mar.1997, 0-year-old: slaughtering age 0~11-month-old: 0 head
- Dec.1996~Mar.1998, 1-year-old: slaughtering age 12~23 : 2
- Dec.1997~Mar.1999, 2-year-old: slaughtering age 24~35 : 27
- Dec.1998~Mar.2000, 3-year-old: slaughtering age 36~47 : 3
- Dec.1999~Mar.2001, 4-year-old: slaughtering age 48~59 : 3

A total of 35 heads

In addition, the number of BSE-cattle which will break out in the future was calculated based on the same assumption described above.

① 1995~96 birth period-cohort

- Considering that 8 years have elapsed since the date of birth (April 1996) of the last detected cattle and the general slaughtering ages (Table.7, Figure.2), most of the steers of this cohort are considered to have already been slaughtered. There is little possibility that other BSE-cattle will be detected in the future.
- Using calculating formula①, in terms of cows, an additional 10

BSE-cattle (9 BSE-cattle have been detected since 2001) are estimated to be detected between 2004~2011.

② 2001~02 birth period-cohort

・ Considering that 30 months have elapsed since the date of birth (January 2002) of the last detected cattle as of July 2004 and the general slaughtering ages of steers (Table.7, Figure.2), 75% of the steers of this cohort are considered to have already been slaughtered, moreover, 99% of the steers of this cohort are predicted to be slaughtered by January 2005, after a lapse of 36 months. Since investigation of the past has detected two 2-year-old BSE-cattle in this cohort, the BSE infection rate of this cohort can be calculated. By multiplying this rate by the number of slaughtered cattle for every slaughtering age(Table.7), the estimated number of BSE-cattle which will be detected in the herd to be slaughtered in the future can be obtained as follows:

{Estimated number of BSE-cattle} = {BSE infection rate of the cohort} × {The number of slaughtered cattle in one year}・・・(②)

In this regard, $\{\text{BSE infection rate of the cohort}\} = \frac{C}{D}$

{C = 2 ＝：The number of BSE-cattle at 2-year-old}

{D =231,502：The number of slaughtered cattle at 2-year-old}

An additional 3 BSE-cattle are estimated to be detected by the end of 2004.

・ Since no BSE-cattle have been detected in cows in this cohort, estimation of BSE-cattle which will be detected in the future is difficult. In this regard, there is a possibility that BSE-cattle will be detected as the number of slaughtered cattle increase with the passage of time according to the number of slaughtered cattle classified by slaughtered ages (Table. 7).

Meanwhile, this estimation hypothesized that BSE-cattle will be detected among ①1995~96, ②2001~02 birth period-cohort. However, the possibilities that BSE-cattle contaminated into the human food chain earlier remains obscure. Some reports speculated that BSE prion

intruded in Japan through the use of imported live cattle from the UK as meal after rendering since the late 1980s[27].

3-3-1-2. Evaluation of vCJD risk in Japan based on the estimation of vCJD patients in the UK simply on a pro-rata basis.

Risk evaluation of vCJD in Japan was carried out based on the presumption (3-2) used for Smith's theory as follows: ① The number of vCJD-patients correlates with that of BSE-cattle at the time when measures were inadequate. ② Considerable individual variation can exist in the incubation period, but it follows a specific statistical distribution. ③ No correlation is assumed between the incubation period and the ages of infection. ④ vCJD only occurs among those whose genotype is M/M at amino-acid residue 129 in the prion protein gene. ⑤ Patients during the incubation period are excluded. ⑥ The relationship between the quantity of prion and the incidence rate is not taken into consideration.

(Estimation 1)

The possible number of vCJD patients in Japan was estimated based on the estimations of BSE-cattle and vCJD patients in the UK. The following hypotheses were proposed: ① An estimated 1 million BSE-cattle were contaminated into the human food chain before the feed ban of MBM on August 1st 1996 in the UK[28),29)]. ② The number of vCJD patients in the UK is estimated at 5000, this is the most pessimistic calculation by Smith et al. In this regard, several reports suggest the number of BSE-cattle in the UK as follows: 2~2.5million, 3.5 million and 4.2million. In this estimation, 1 million, the smallest number, was adopted to maximize the estimated risks. Meanwhile, vCJD patients in Japan can be estimated on a pro-rata basis by applying the correlation between the estimated number of BSE-cattle contaminated into the human food chain in the UK (1 million) and that of vCJD patients (5,000) caused by this. Given 5 BSE-cattle were contaminated into the feed chain in Japan as described previously, the estimation on a pro-rata basis is as follows:

$$5{,}000 \text{ patients} \times \frac{5 \text{ BSE-cattle}}{1{,}000{,}000 \text{ cattle}} \quad \cdots (③)$$

In addition, considering the ratio of people carrying M/M at codon 129 in the prion protein gene (UK; 40%, Japan; 90%) in the national population of both countries (UK; 50million, Japan; 120million).

$$③ \times \frac{(120\ \text{million} \times 90\%)}{(50\ \text{million} \times 40\%)}$$

As a result, 0.1 is the estimated number of vCJD patients of the whole population in Japan by BSE prion uptake before the implementation of testing all cattle.

In addition, in the UK, mechanically recovered meat (MRM) is considered as one of the major causes since it raises the possibilities of contamination of the brain and spinal cord. Given the fact that MRM has never been used in Japan, however, the infection risks will be estimated much lower.

(Estimation 2)
In the same manner, the number of vCJD patients in Japan is predicted at 0.9 by applying 35, as the number of BSE-cattle having entered the human food chain without detection in the past, to the formula③ described above and calculate on a pro-rata basis followed by correction by the composition ratios of population and genotype.

However, the actual value would be predicted to be lower considering the facts that infectivity becomes higher in a host with age, and BSE-cattle which are supposed to be contaminated into the human food chain in estimation 2 are made largely of younger cattle in the 2nd year after infection.

3-3-2. Risk reduction through control measures

In Japan, as countermeasures against BSE, SRM removal in slaughterhouses and BSE test have been performed to protect consumers'

health since September-October 2001, and import of beef materials as various ingredients in medicines have been banned from countries where BSE occurred, at the same time, its use for commercial products have been prohibited. Meanwhile, as measures against BSE occurrence, management of feed production, regulations imposed on usage of feed, introduction of traceability, and testing cattle with neurological symptoms, dead cattle, and cohort cattle (hereinafter called risk cattle) for surveillance have been introduced. Among them, SRM removal in slaughterhouses and BSE tests are considered to have greatly contributed to directly lower the risk of BSE infection to humans.

3-3-2-1. Countermeasures against BSE occurrence

This document aims to estimate the infection risk of BSE prions from cattle to humans in ordinary eating habits. At the same time, in order to eradicate BSE, the following measures are required: management and regulation of feed, introduction of traceability, and testing risk cattle. Especially, the regulation of animal feed is regarded as a fundamentally essential measure to prevent BSE infection and to guarantee the reduction of risk of BSE infection from cattle to humans.

- **Management and regulation of animal feed**

  In September 2001, when the first BSE-cattle was discovered in Japan, in order to implement measures based on “Legislation for securing feed safety and improving feed quality”, feeding ruminant animals by MBM derived from ruminant animals was prohibited. In October 2001, usage of MBM for feed has been totally prohibited. By these prohibitions, in theory, transmission of BSE prions from cattle to cattle is presumed to have been completely blocked off. However, securing effectiveness of feed regulations is required from now on since the possibilities of cross-contamination in the process of feed production in feed mixture factories and during transportation of materials have been pointed out, regarding up to the 7th BSE-cattle detected in Japan, by the Epidemiological Surveillance Team of the Ministry of Agriculture, Forestry and Fisheries of Japan, and since causes are still unidentified by epidemiological studies and the possibility of cross-contamination of BSE prions can not be denied regarding the 8th and 9th BSE-cattle which

were born after October 2001.

・**Implementation of the traceability system**

In Japan, since December of last year, based on the “Special measures law on management and transmission of information for individual recognition of cattle”, the traceability system has been made compulsory to document information including birth record, which allows individual recognition at the production and slaughtering stages and determination of accurate ages. This allows separate investigation of the degree of risk of BSE infections before or after implementation of the various regulations.

In addition, this regulation has also been implemented at the distribution level since December of this year. Taking into account its significance to create transparency for consumers to directly obtain information of beef, securing and verification of the traceability system are deemed essential from now on.

・**Testing risk cattle**

With regards to testing risk cattle, to comprehend the extent of BSE infection in Japan and validate the effectiveness of measures against BSE, the first surveillance covering cattle at 24-month-old or older was implemented before the 1st BSE-case was identified in Japan. Moreover, since October 2001, dead cattle were added to the surveillance and, since April 2004, screening of all dead cattle at 24-month-old or older has been implemented. Until now, 69218 cattle have been examined and one BSE-cow was detected. (Statistics of the Ministry of Agriculture, Forestry and Fisheries of Japan; October 18th 2001~May 31st 2004)

Testing risk cattle should be continued in the future.

3-3-2-2. Risk reduction through BSE test, its detection limit and significance

The BSE test is carried out by slaughtering inspectors, who own veterinarian's licenses and civil servants in slaughterhouses of prefectural and city governments based on “Legislation for slaughterhouses” and “Special Measures Law on Bovine Spongiform Encephalopathy”. And BSE tests are significant in the following two ways: ① Exclusion of BSE-cattle

from the human food chain ② Comprehension of the extent of BSE contamination and verification of the effectiveness of measures against BSE in Japan.

Exclusion of BSE-cattle from the human food chain means reduction of risk of BSE infection by consuming BSE prion-contaminated beef and internal organs and directly contributes to the protection of consumers' health. Based upon results of the BSE test, 9 BSE-cattle have been identified and were eliminated from consumption. Among them, 21 to 23-month-old cattle were involved, of which the detection was lead by testing all cattle. Thus, this suggested the effective contribution of BSE test to protect the consumers' health.

In Japan, as a result of the introduction of testing of all cattle implemented as the cattle were processed for consumption soon after occurrence of BSE, valuable data regarding these cattle were obtained to help speculate on the BSE infection status within a short time. With respect to the BSE contamination status in the field, accumulation of data obtained from testing dead cattle at 24-month-old or older, which has been fully implemented in farms since April of this year, will improve the accuracy of epidemiological information based on the condition of BSE contamination in Japan.

- **Detection limit of BSE prions by rapid test**
  The accuracy of BSE test methods employed as a primary test in Japan, "Prateria BSE"(Biolad) and"Dinabot Enfar BSE"(Dinabot), is being evaluated by Scientific Steering Committee in European Comission[30]. "Prateria BSE" possesses virtually equal reliability compared with the mouse-bioassay and its detection limit is 2M.i.c.ID_{50}/g (M.i.c.ID_{50}: 50% infectivity after inoculating into the mouse brain). Therefore, accumulation of abnormal prion proteins greater than the detection limit （≧2M.i.c.ID_{50}/g） will not be tested negative but definitely positive. And in cases where the amount of abnormal prion proteins is below the detection limit, it will be tested negative. In other words, BSE-cattle in the incubation period with infectivity below 2M.i.c.ID_{50}/g in the medulla oblongata (obex) will be tested negative.

"Dinabot Enfar BSE" as well as "Prateria BSE" were reviewed by the Pharmaceutical Affairs and Food Sanitation Council and their import as veterinary diagnostic products was approved by the Minister of Agriculture, Forestry, and Fishery. Moreover, it was reviewed under the evaluation result of the European Commission by the "Expert Committee for Bovine Spongiform Encephalopathy (BSE) Diagnosis" held by the Ministry of Health, Labour and Welfare and approved its sensitivity equal to that of "Prateria BSE."
These facts prove the contribution of BSE test at slaughterhouses to risk reduction of BSE infection in humans caused by consuming beef and internal organs, but it cannot be concluded that present testing methods can detect and exclude all the BSE-cattle during the incubation period because of their technical limits.

- **Detectable age by rapid test**

No experiments for this purpose have ever been performed, just the fragmentary facts described below are known.
In the experiment in the UK described above, infectivity was first confirmed ($\leqq 10^{3.2} \sim 10^{5.6}$ C.i.c.ID_{50}/g) in the cattle brains at 32 months after oral administration and no infectivity was confirmed in the experimentally infected cattle at 22~26 months after administration in 4-month-old cattle. These results mean that the accumulation of abnormal prion protein at the medulla oblongata (obex) greater than the detection limit first occurs at 32 months after administration.

Meanwhile, in Japan, 21 to 23-month-old BSE-cattle were identified among 9 BSE-cattle confirmed by testing of approximately 3.5million cattle at slaughterhouses. Notably, results of the WB method demonstrated the amount of abnormal prion protein contained in the medulla oblongata (obex) was small, presumably from 500 to 1000 times less than in other BSE-cattle found in Japan[1)]. Therefore, detection of BSE-cattle at 20-month-old and below by the current sensitivity of the testing method is deemed difficult.
In addition, the youngest BSE-case on the present record is 20-month-old cattle found in the UK in 1992. A scientific report prepared by the TSE/BSE ad hoc group of the European Commission speculated that

infectivity can be detected as early as 17-month-old in the case in which BSE disease onset is confirmed at 20-month. This was based on the data obtained by an infection experiment carried out in the UK which demonstrated that infectivity was detected in the 32nd month after administration and BSE development was confirmed in the 3rd month after detection of infectivity. In this regard, this British case cannot immediately be applied to measures against BSE in Japan because of the enormous difference regarding BSE contamination status and the amount of exposure of cattle to BSE prions in the UK compared with those in Japan.

- **Prospects of test**

  Investigations for improvement and development of rapid BSE test methods are being performed in European countries, the U.S., and Japan and rapid testing methods with greater sensitivity are expected to become available. It is considered that the lower the detection limit is, the younger the detectable BSE-cattle will be. Moreover, demonstration of the presence or absence of infection before slaughtering can be expected if testing using tissues or blood samples removed from live cattle becomes available. This will allow the detection and exclusion of BSE-cattle without bringing them into slaughterhouses and incurring the risk of SRM cross-contamination, and lead us close to the goal described in the report[3)] by the Scientific Steering Commission in Europe: Exclude infected animals from the human food chain to protect consumers from the risk of BSE infection.

  Studies to improve the BSE testing method should be continued including improving the detection limit, and further investigation is required for quantitative evaluation of the risk originating from cattle at 20-month-old or younger.

3-3-2-3. Risk reduction by SRM (specified risk materials) removal

- **SRM removal**

  In Japan, use of cattle heads (excluding the tongue and cheek flesh), spinal cord, distal part of ileum, spinal column including dorsal root ganglion for food products is prohibited for all cattle by law. Since current findings indicate that more than 99% of abnormal prion protein

concentrate in these tissues, complete exclusion of these tissues from the human food chain is expected to practically reduce the vCJD risk in humans.

However, it is not practical to consider that complete SRM removal is implemented in slaughterhouses because of the residual spinal cord left behind during spinal cord removal, possible contamination of dressed carcasses, and contamination of central nervous tissue by pithing.

The results of surveillance concerning the removal rates of spinal cord before back-spreading in 7 Meat Hygiene Inspection Offices directed by the Ministry of Health, Labour and Welfare are as follows: 5 offices employing the spinal cord aspiration method indicated average 80.6± 17.1%(52.5~99.1%), and 2 offices employing the spinal cord extrusion method indicated average 75% (72.0, 78.0%). In addition, the residual spinal cord after back-spreading was disposed of manually[32].

Absence or presence of tissues other than SRM in which abnormal prion protein accumulates cannot be determined at this point because of the detection limit of the infection experiments by which SRM was identified and uncertainty derived from incomplete understanding of mechanisms underlying BSE infection. These ideas are supposed to be the grounds on which the World Health Organization is recommending exclusion of any BSE-cattle tissue from the human food chain.

- **Contamination during slaughtering**

  Surveillance by the Ministry of Health, Labour and Welfare described above, to examine the presence or absence of contamination of spinal cord tissues in the dressed carcasses by wiping using a detection kit, showed no significant differences according to the spinal cord removal methods: spinal cord aspiration method, spinal cord extrusion method, and spinal cord removal method after back-spreading[32].

  So-called pithing, the destruction of brain and spinal cord with wire in slaughtering, has been prohibited in the EU since 2000 in response to reports which demonstrated the migration of central nervous tissue to other tissues via the blood stream with some slaughtering methods[34].

Meanwhile, in Japan, the Ministry of Health, Labour and Welfare is guiding industry officials involved to prohibit pithing because of concerns about contamination by leaks of brain and spinal cord tissues and difficulties of effective sterilization of metal-wire used for each cattle, but pithing is not prohibited based on the situation that forces its use from the viewpoints of work safety. In addition, according to the examination concerning the leakage of central nervous tissues into blood performed by the Ministry of Health, Labour and Welfare, it cannot be concluded that pithing contaminates blood with brain and spinal cord tissues, but there is a possibility that employment of pithing contaminates meat and slaughterhouse facilities through leakage of brain and spinal cord tissues from the stunning hole[35]. Given these results, in the future, further review is required concerning the management of pithing including its prohibition. In addition, these days, possible contamination of carcasses is pointed out by the use of stun guns.

BSE test in slaughterhouses is considered to be playing a major role to reduce the risk of SRM contamination in slaughtering.

3-3-3. Current risks

(Estimation 1)

In the future, with regard to the scale of BSE occurrence in Japan, there is a possibility that a maximum 60 BSE-cattle will be confirmed by 2005, 6.

However, the risk of humans being infected with vCJD by BSE-cattle through the human food chain is presumed to be mostly eliminated by the current SRM removal and BSE test as long as they are implemented appropriately.

(Estimation 2)

The predicted number of occurrence of BSE-cattle in the future is as follows: ① From 2004~11, 10 BSE-cattle will be confirmed in cows of 1995~96 birth period cohort (At present, 9 BSE-cattle have been confirmed since 2001.) ② By the end of 2004, an additional 3 BSE-cattle, a total of 13, are presumed to be confirmed in cattle of 2001~02 birth period cohort. But the risk of humans being infected by BSE-cattle through the human food chain is

presumed to have been mostly eliminated as far as the current SRM removal and BSE tests are appropriately implemented.

3-3-4. Increase and decrease of the risk in accordance with the selection of management measures

Among measures against BSE implemented in Japan, SRM removal at slaughterhouses and BSE tests contribute greatly to the reduction of BSE infection to human beings through consumption of beef and the internal organs of cattle.

Above all, SRM removal covering all cattle is considered as an effective management measure at present, so the system should be continued.

In addition, with regard to BSE test at slaughterhouses, even if cattle infected below the level of detection threshold are excluded from BSE test, it will not affect the detection of BSE-cattle by the test and the risk of BSE infection to humans will not increase by entering BSE-cattle into the human food chain.

However, there is only fragmentary data available to date concerning when those cattle which carry abnormal prion proteins at around the detection limit become detectable, that is, at what stage of incubation period or at what age. From the fact that two 21-and 23-month-old cases have been identified among 9 cases discovered by testing approximately 3.5 million cattle in Japan, there is a possibility that the presence of BSE prions may be detected by current diagnosis in cattle at the age of 21-months and over.

Studies to improve the BSE testing methods should be continued, taking into account the possibility that the detection limit will be improved.

Based on the studies, experiments for quantitative risk evaluation should be continued, and in addition efforts aimed at collecting and reviewing information such as data obtained from oral administration experiments which are in progress in Japan and other foreign countries should be promoted.

4. Conclusions

(1) In the future, additional BSE cases are likely to be found in Japan. However, the risk of humans being infected by BSE-cattle through the human food chain is presumed to be effectively eliminated by the current SRM removal and BSE test.

(2) As long as the current measure of SRM removal from cattle of all ages remains unaltered, the risk of vCJD infection should not increase, even if cattle infected below the level of the detection limit are excluded from BSE test. However, there is not sufficient information available to date regarding when those cattle which accumulate abnormal prion protein at around the detection limit in the medulla oblongata (obex) become detectable, at what stage of incubation period, or at what age. From the fact that two 21-and 23-month-old cases have been identified among 9 cases discovered by testing approximately 3.5 million cattle in Japan, there is a possibility that the presence of BSE prions may be detected by current diagnosis in cattle at the age of 21-months and over.
However, the amount of abnormal prion proteins detected by the WB method in the 21-and 23-month-old cases was 500 to 1000 times less than in the other cases. Moreover, no case at the age of 20-months or younger has been found by testing approximately 3.5 million cattle in Japan. These facts should be fully taken into account in considering the future measures against BSE in Japan.

(3) Studies to improve the BSE testing method should be continued, including improving the detection limit and developing an ante-mortem testing method using tissues or blood from live cattle. Further investigation is also required for quantitative evaluation of the risk originating from cattle at 20 month-old and below.

(4) Since current data suggest that more than 99% of abnormal prion protein in cattle developing BSE were found in SRM, SRM removal is considered as an extremely effective measure to reduce the risk of BSE infection to humans. In addition, with regard to prevention of cross-contamination, because brain tissue of as little as 0.001~1g from infected cattle, reportedly becomes a possible source of infection,

prevention of cross-contamination through appropriate slaughtering and processing in slaughterhouses is important to reduce the risk of BSE infection to humans. For this reason, adequate schemes should be established to ensure the implementation of thorough SRM removal, and prevention of cross-contamination, as well as periodical monitoring of their implementation.

(5) The risk of a BSE epidemic is considered extremely low because of the current regulations imposed on animal feed stuff as a countermeasure. However, inspection by the authorities to ensure their effectiveness must be continued, considering the fact that young BSE cases have been detected. In addition, securing and verification of the traceability system and continued implementation of BSE test of risk animals are essential.

5. Closing remarks

The BSE issue is one which is attracting the most attention from people and has a great impact on society in issues of food safety and security. Meanwhile, it's a fact that BSE is a disease of which many parts remain scientifically unsolved. Measures against the BSE issue which shows manifold aspects and uncertainties are expected to be determined through thorough risk communication with people by risk regulating agencies; risk regulation and risk communication should be based on the recognition that protection of public health has a top priority.

In addition, more surveillance studies by the Ministry of Health, Labour and Welfare and the Ministry of Agriculture, Forestry and Fisheries of Japan should be promoted to clarify uncertainties which remain to be elucidated scientifically, and quantitative risk evaluation must be performed based on the data and findings obtained from these efforts.

(Abbreviations)

BSE	Bovine Spongiform Encephalopathy
vCJD	variant Creutzfeldt-Jakob Disease
OIE	Office International des Epizooties
ID_{50}	50% infective Dose
C.i.c.	Intracerebral inoculation in the cattle brain

C.o.	Oral administration in the cattle
M.i.c.	Intracerebral inoculation in the mouse brain
SRM	Specified Risk Materials
WHO	World Health Organization

(References)

1 Yamakawa Y, Hagiwara K, Nohtomi K, Nakamura Y, Nishijima M, Higuchi Y, Sato Y, Sata T and the Expert Committee for BSE Diagnosis, Ministry of Health, Labour and Welfare of Japan. Atypical Proteinase K-Resistant Prion Protein(PrP^{res}) Observed in an Apparently Healthy 23-Month –Old Holstein Steer. *Jpn. J. Infect. Di*s. 2003; 56

2 Scientific Steering Committee. Listing of Specified Risk Materials: a scheme for assessing relative risks to man, Opinion of the SSC adopted on 9 December 1997

3 Scientific Steering Committee. Opinion on the Scientific Steering Committee on the human exposure risk (HER) via food with respect to sBSE, Adopted on 10 December 1999

4 Scientific Steering Committee. Opinion on TSE Infectivity Distribution in ruminant Tissues (State of Knowledge, December 2001), Adopted by the Scientific Steering Committee at its meeting of 10-11 January 2001.

5 Wells GA, Hawkins SA, Green RB, Spencer YI, Dexter I, Dawson M. Limited detection of sternal bone marrow infectivity in the clinical phase of experimental bovine spongiform encephalopathy (BSE). *Vet Rec*. 1999;144(11): 292-294.

6 Spongiform Encephalopathy Advisory Committee. Statement on Infectivity in Bovine Tonsil. Jan 2002

7 Wells GA and Wilesmith JW. The neuropathy and epidemiology of bovine spongiform encephalopathy. *Brain Pathol.* 1995; 5; 91-103.

8 Office International des Epizooties: Bovine spongiform encephalopathy, Manual of standards for diagnostic tests & vaccines, Chapter 2.3.13, (2001)

9 Grants for Health Science 2002, Multidisciplinary study for Securing of Health and Safety, Research for Emergency Plan to Overcome Hepatitis etc.（Bovine Spongiform Encephalopathy）”Studies for Improvement of Prion Detection Methods and infection/development mechanisms of

Bovine Spongiform Encephalopathy" Chief Scientist Sata Tetsutaro, Director of Infectious Disease Pathology of National Institute of Infectious Disease

10 Neil A. Mabbott and Moiria E. Bruce: The immunobiology of TSE disease. *J Gen Virol* 2001; 82:2307-2318

11 Federal Institute of Risk Assessment（BfR）: Expression of the opinions relevant to raising the age of BSE inspection of slaughtered cattle from 24-month-old or older to 30-month-old or older. December 22 , 2003

12 Scientific Steering Committee. Opinion Oral Exposure of Human to The BSE Agent: Infective Dose and Species Barrier Adopted by SSC at its Meeting of 13-14 April 2000 Following a Public Consultation via Internet between 6 and 27 March 2000

13 Collinge J, Palmer MS, Dryden AJ. Genetic predisposition to iatrogenic Creutzfeldt-Jacob disease. *Lancet* 1991; 337: 1441-1442.

14 Owen F, Poulter M, Collinge J, Crow TJ. Codon 129 changes in the prion protein gene in Caucasians. *Am J Hum Genet.* 1990; 46(6):1215-1216.

15 Windl O, Dempster M, Estibeiro JP, Lathe R, de Silva R, Esmonde T, Will R, Springbett A, Campbell TA, Sidle KC, PalmerMS, Collinge J. Genetic basis of Creutzfeldt-Jacob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the PRNP gene. *Hum Genet.* 1996; 98(3):259-264.

16 Soldevila M, Calafell F, Andres AM, Yague J, Helgason A, Stefansson K, Bertranpetit J. Prion susceptibility and protective alleles exhibit marked geographic differences. *Hum Mutat.* 2003; 22(1):104-105.

17 Doh-ura K, Kitamoto T, Sakaki Y, Tateishi J. CJD discrepancy. *Nature.* 1991; 353 (6347):801-802.

18 Peden AH, Head MW, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. *Lancet* 2004;364:527-529

19 Hilton DA , Ghani AC, Conyers L, Edwards P, McCardle L, Ritchie D, Penney M, Hegazy D, Ironside JW. Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *J Pathol.* 2004; 203(3):733-739.

20 Llewelyn CA, Hewitt PE, Knight RSG, Amar K, Cousens S, Mackenzie J, Will RG. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004;363: 417-421.

21 'Aignaux JN, Cousens SN, Smith PG. Predictability of the epidemic of variant Creutzfeldt-Jacob disease by back-calculation methods. *Stat Meth Med Res* 2003; 12:203-220.
22 P Smith. The epidemics of BSE and vCJD in the UK（6th Prion Expert Committee Document, Presentation-slide by Mr. Peter Smith）
23 'Aignaux JN, Cousens SN, Smith PG. Predictability of the UK variant Creutzfeldt- Jacob disease epidemic. *Science.* 2001; 294(5547):1729-1731.
24 Yoshikawa. Unpublished data
25 Ministry of Agriculture, Fishery, and Forestry in Japan：Reports on results of epidemiological survey by BSE Epidemiological Investigation Team. September 2003
26 Yoshikawa. 9th Report 2004 Prion Expert Committee
27 Sugiura K. Risk of introduction of BSE into Japan by the historical importation of cattle from the United Kingdom and Germany. *Preventive Vet Med* 2004; 64:191-200.
28 Donnelly C.A. and Ferguson N.M. Statistical aspects of BSE and vCJD: models for epidemics. *Monographs on Statistics and Applied Probability No.84. Chapman & Hall/CRC, Boca Ratonm Floroda.* 1999: 256pp
29 Ferguson N.M., Donnelly C.A., Woolhouse M.E.J, Anderson R.M. The epidemiology of BSE in Cattle herds in Great UK. II. Model construction and analysis of transmission dynamics. *Philos. Trans. Roy. Soc. Lomd., B, Biol. Sci.* 1997; 352(1355): 803-838)
30 Scientific Steering Committee. The Evaluation of Tests for The Diagnosis of Transmissible Spongiform Encephalopathy in Bovines, 8 July 1999
31 Scientific Steering Committee. Scientific Report on Stunning Methods and BSE risks (The risk of dissemination of brain particles into the blood and carcass when applying certain stunning methods.) prepared by the TSE BSE ad hoc Group at its meeting of 13 December 2001 and including the outcome of a public consultation via Internet between 10 September and 26 October 2001.
32 2001 Health Science Research Grants （Special Research Project for Health Science） Review of Research Reports “Studies of Bovine Spongiform Encephalopathy (BSE)” Chief Scientist Morikazu Shinagawa Laboratory of Veterinary Public Health, Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary medicine.

33 World Health Organization: Report of a WHO Consultation on Public Health Issues related to Human and Animal Transmissible Spongiform Encephalopathies. WHO/EMC/DIS/97.147 Genva, Switzelarland, 2-3 April 1996.

34 Anil MH, Love S, Williams S, Shand A, McKinstry JL, Helps CR, Waterman-Pearson A, Seghatchian J, Harbour DA. Potential contamination of beef carcasses with brain tissue at slaughter. *Vet Rec.* 1999; 145(16):460-462.

35 2001 Health Science Research Grants for Health Science 2002, Multidisciplinary study for Securing of Health and Safety, Research for Emergency Plan to Overcome Hepatitis etc. （Bovine Spongiform Encephalopathy）"Studies for Improvement of Prion Detection Methods and infection/development mechanisms of Bovine Spongiform Encephalopathy" Chief Scientist Sata Tetsutaro, Director of Infectious Disease Pathology of National Institute of Infectious Disease Reports on shared research "Research to Prevent Contamination by Beef that Contains Nerve tissue"

36 Prendergast DM, Sheridan JJ, Daly DJ, McDowell DA, Blair IS. The use of a marked strain of *Pseudomonas fluorescens* to model the spread of brain tissue to the musculature of cattle after shooting with a captive bolt gun. *J Appl Microbiol*. 2004; 96(3):437-446.