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Clinical Epidemiologic Evaluation of BSE Screening Tests in Japan

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To address the public concerns provoked by the first incidence of bovine spongiform encephalopathy (BSE) in Japan, the BSE screening tests in Japan are evaluated in use of modeling analysis in evidence-based diagnosis.

Under the assumptions based on epidemiological statistics such as the annual number of screened cattle with 1,227,385, the annual incidence of BSE infection with four, and the sensitivity of 99.0% for both primary and secondary tests, it was estimated that, the current threshold of cut-off for the BSE positive would have 0.119 false negatives per year. The decrease of the sensitivity of ELISA down to 90.0 % resulted in the increase up to 0.792 false negatives per year. Even with the 90.0% sensitivity, shifting the cut-off point from the current level to the best one remarkably reduced the false negatives per year down to 0.0004. Regarding false positives, with 99.7 % specificity for both ELISA and the confirmatory tests revealed the risk of 0.03 false positives per year, while the cut-off shifting that can best minimize false negatives largely increased the false positives up to 11,013.

Although it is confirmed the possibility of false negatives is very low, the current method of screening can be further improved by shifting the decision level of cut-off to define the BSE “positive”. Such an improvement, however, raises an issue of trade-off : the less false negatives, the more false positives. We believe our approach can help the public perception of an optimum decision-making for BSE screening, considering the trade-off.

The first case of bovine spongiform encephalopathy (BSE) was identified in the United Kingdom in 1986. Since the early stage, the disease has been recognized as a potential threat to the world. In 1991, for example, as a result of a meeting arranged by the World Health Organization (WHO), “Report of a WHO Consultation on Public Health Issues Related to Animal and Human Spongiform Encephalopathies” outlined recommendations, in which not only BSE but also its human counterpart was covered.

Nonetheless, in the beginning, the disease’s transmissibility to human species was not scientifically confirmed. On the contrary, it was generally considered to affect only livestock at the time. In addition, it was deemed that the disease would be eradicated by the year 2000 (11). Nevertheless, in 1996, 10 cases of variant Creutzfeldt-Jacob Disease (vCJD), inferred to have been caused by the intake of BSE infected beef (or its nervous system), were reported, and, in turn, transmissibility from cows to humans was brought to

attention. Eventually, the “mad cow disease” became a major social concern affecting not only livestock, but also people—i.e., consumers as well as producers of beef in the world.

In Japan, the first cow infected with BSE was found in 2001. After the first case, the general public’s reaction and concern about the disease became rather extraordinary and excessive, as previously seen in some other countries (13). As countermeasures, the Japanese government adopted a series of screening tests of all cattle that would be consumed by humans, and, in addition, made a “safety declaration” assuring that any BSE-affected beef would not be sent out to the market (9). This declaration, however, did not subside people’s anxiety about the extent of safety and risk, and brought about the recognition that people were in fact seeking objective information about the validity of the testing procedures and the level of infectivity or transmissibility to humans.

The government in Japan thereafter implemented BSE-related policies involving risk analysis, and in September 2004 informed the public about vCJD. According to the information provided by the government, a correlation between the risk of vCJD and the number of BSE-infected cattle was suspected. The focus of the information was placed on the incidence. Subsequently, the first death resultant of vCJD occurred in the country in December 2004. This brought about a resurgence of interest and concern over BSE and its incidence. Yet, to say the least, scientific discussions on the screening tests have not been carried out sufficiently.

By and large, the BSE tests in Japan involve two distinct stages for primary and confirmatory screening. The first stage is composed of two consecutive Enzyme-Linked Immunosorbent Assay (ELISA), and the second stage involves the Western blot method and immuno-histochemical test (Fig. 1). In this sequence of tests, samples found to be positive in both of the two ELISA tests in the first primary screening are forwarded to the secondary or confirmatory screening. In contrast, those determined negative in the first ELISA are presumed to be ready for being sent to the market. In addition, there is a possibility that cows which show negative results in the confirmatory tests be subsequently taken to the slaughterhouse and then to the market in spite of the positive result in the primary stage. In other words, in the current situation, all except those that are detected positive through the cut-off point CP1 (Fig. 1) are to be sent to the slaughterhouse and thereafter supplied in the market as beef for human consumption. Thus, the current screening procedures have the risk of letting false negatives, or the cattle that do in fact have BSE but falsely show negative results, be in the beef market. In consequence, those beef might subsequently be consumed by humans. That is a serious concern to the public.

Accuracy of a diagnostic test is influenced and determined by the sensitivity and the specificity. In this context, the sensitivity refers to the probability of correctly identifying a BSE-infected cow as positive, and the specificity, on the other hand, represents the probability of rightly determining a BSE-free cow as negative. In order to assess the BSE screening in Japan in terms of the extent of the diagnostic accuracy, it is important to analyze quantitatively the behavior of current screening system with certain sensitivity and specificity. From the standpoint of consumer protection, the serious problem lies in false negatives, whose indicator is the sensitivity of the diagnostic tests. A false positive rate, deriving from the specificity, is the probability of falsely determining positive by the test among BSE-free cows, and hence the concern about the error on false positive is a problem only for producers of beef, but not for consumers. That is, the sensitivity is more important for consumers than the specificity in terms of the former’s direct relation to minimizing the risk of overlooking true BSE cases. Actually, when keeping the specificity at a constant level, a higher sensitivity level would, in turn, lead to having a smaller number of ‘negative’ cases

EVALUATION OF BSE SCREENING IN JAPAN

FIG.1. The modes and sequence of BSE testing

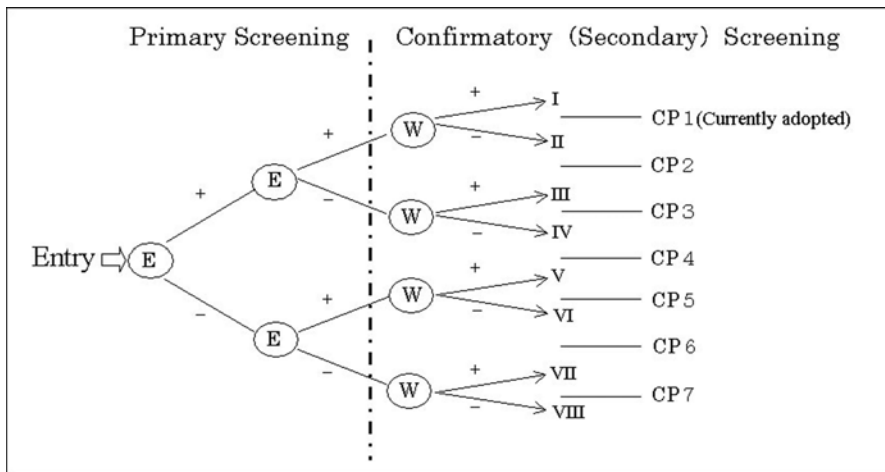


FIG.1. The modes and sequence of BSE testing

“E” stands for Enzyme-Linked Immunosorbent Assay (ELISA), while “W” is the abbreviation for Western blot and immuno-histochemical tests. In the Primary Screening, ELISA is carried out twice. Western blot test and immuno-histochemical test are conducted once during the Confirmatory or Secondary Screening. This entire sequence will lead each cow to one of the eight endpoints, from I through VIII. CP1 through CP7 are cut-off points to determine and differentiate non-infected and infected cows. The current screening system in Japan employs CP1, whereby “I” is treated as BSE-infected and “II” through “VIII” are treated as non-infected.

detected by the test among BSE-infected cattle. Kamae et al (7). addressed such an issue for assessing the current BSE screening, focusing on false negatives, and reported that even if Japanese cattle were in a low risk group, a few of them are likely to be misdiagnosed as negative in spite of true BSE under the current screening system. In the analysis by Kamae et al., however, the question remained undeveloped: how we could quantitatively evaluate the trade-off between false positive and false negative, which would be the trade-off of social and medical impacts between producers and consumers of beef, depending on the decision regarding cut-off points in the BSE screening system. In this study, therefore, the focus is mainly placed on the trade-off issues as follows:

- 1) how many false negatives will be estimated depending on different levels of cut-off in the screening such as CP1 through CP7 shown in Figure 1
- 2) how the number of false negatives overlooked by the current system will vary as the sensitivity changes under uncertainty

METHODS

In this study, the possibility of false negative outcomes in the BSE screening tests were examined, using computing simulations. For that purpose, we assumed target population for screening, prevalence of BSE, and accuracy of the tests to set them as parameters for the simulation.

Assumptions

- 1) Target population for screening

In the 42-month period from October 2001 to March 2005, the total of 4,295,849 cattle have been tested, and 12 have been diagnosed as having contracted BSE (10). Therefore, there would be 1,227,385 cattle $(= (4,295,849/42) \times 12 = 1,227,385)$ to be screened annually.

2) Prevalence of BSE in cows

In Japan, as of October 2005, there is no reliable data available on BSE prevalence. Instead, it is estimated that 3.4 cows would be infected with BSE per year, according to the detected BSE cows of 12 in 42 months. This estimate does not include unknown cases of false negatives, and treat only those cattle determined positive in the screening tests as infected. In order to minimize the possibility of underestimating such a risk, this study adopts the value of four as the true cases of BSE per year. Accordingly, the number of cows not infected with BSE per year would be 1,227,381 (=1,227,385-4).

3) Accuracy of the tests

According to the European Commission (EC), the currently-applied kit for the ELISA has both the sensitivity and the specificity of 100 percent (3). With respect to the 95 percent confidence interval (95CI), the lower limits are 99.0 percent and 99.7 percent for the sensitivity and the specificity, respectively. In one study conducted in March 2002, another kit of ELISA was tested for its sensitivity and specificity, and the results were found to be 97.9 percent for the sensitivity and 100 percent for the specificity (4). As for the 95 CI, the lower limit of each turned out to be 90.1 percent and 98.0 percent. Moreover, according to the report in November 2004, all kits applying ELISA have 100 percent sensitivity and specificity, while at the 95CI, the lower limits were 94.0 percent for the sensitivity and 98.0 percent for the specificity (5). Practically, however, the assumption that the probability of both false negatives' and false positives' occurrences to be completely zero is rather unrealistic and dangerous in terms of risk assessment. In addition, it is important to take into consideration the fact that the aforementioned findings are based on the data in the study where clear and facile differentiation was possible between non-infected and infected BSE cows. Furthermore, in the course of the actual test, there is also a possibility of human errors.

Thereby, in the simulations employed in this study, the sensitivity for ELISA is set between 90.0 and 99.0 percents for the worst and best estimates. On the other hand, for estimating false positives, ELISA's specificity was set at 99.7 percent and 98.0 percent for the lower limits at the 95 CI based on the above-mentioned reports by European Commission (3, 4, 5).

It was reported in 1999 that both the sensitivity and the specificity of the Western blot test were 100.0 percent, and their lower limits at the 95 CI were 99.0 percent and 99.7 percent, respectively. In order to reconfirm the results obtained through the Western blot test, additional immuno-histochemical testing is also employed in the current system. Therefore, we adopted the lower limit of the sensitivity of 99.0 percent and the specificity of 99.7 percent in this simulation so as to consider the worst scenarios just in case.

Model for simulation

All the possible combinations of binary outcomes, "+" or "-", in the BSE screening can be represented in Figure 1. Initially, 4 BSE-infected cows enter the cascade of testing system, and then the expected number of the cows which will get to each node "E", "W", and endpoint I through VIII can be estimated according to the sensitivity of each test. The simulation ends at the points I through VIII. The letter "E" stands for the Enzyme-Linked Immunosorbent Assay (ELISA), whereas "W" is the abbreviation for Western blotting and immuno-histochemical tests. The endpoints are shown as "I" through "VIII." The cut-off points CP1 through CP7 represent decision levels to judge whether a given cow is BSE-infected or not depending on the results of the tests. Currently, the cut-off point with CP1 is adopted in Japan. The CP1 treats only "I" as BSE-infected, but "II-VIII" as not infected.

EVALUATION OF BSE SCREENING IN JAPAN

Sensitivity analysis

To comply with the second question on the trade-off raised in Introduction, sensitivity analyses were conducted along with the change of sensitivities in ELISA by being shifted through six different levels, ranging from 0.900 to 0.990, resulting in estimating the number of false negative cows at each level.

RESULTS

As a result of the numerical simulation, the estimates were obtained as shown in Table 1. Under the hypothetical conditions, in which the sensitivity of 99.0 percent for ELISA and the sensitivity of 99.0 percent for the confirmatory test were assumed, the current cut-off point (CP1 in Fig. 1) identified 3.88 cases per year as positive out of four BSE cows. Figure 2 illustrates the process of numerical simulation in the case of 99.0 percent sensitivity at any test. The sum of the estimates at the endpoints II through VIII, i.e., 0.119, implies cumulative number of false negative by CP1 in Table 1. That is, about 0.12 cattle per year (i.e., one cow every 8.4 years) were falsely identified as negative despite the actual presence of the disease. As used in European Commission's report in 2004 (4), the sensitivity of 94.0 percent brought about the false negative of 0.50, in other words, approximately one false negative cow every two years.

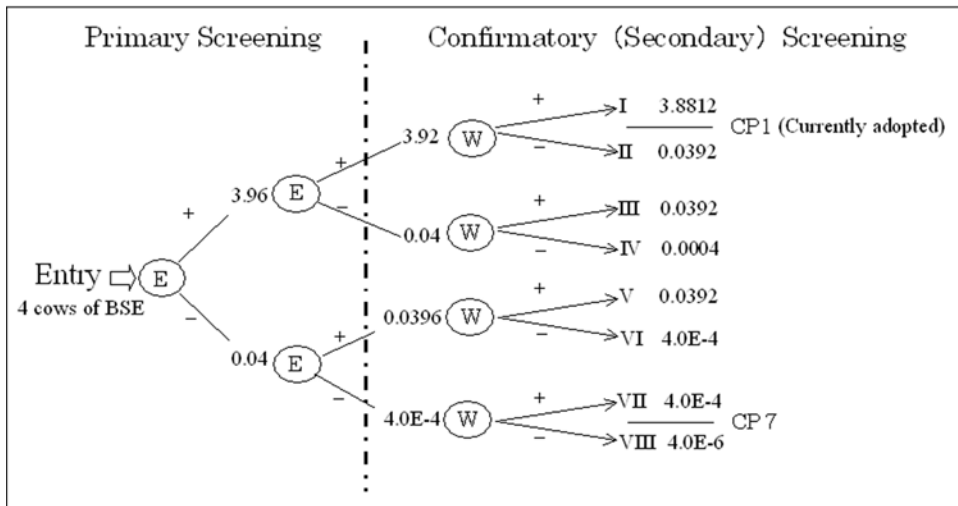
TABLE1. Estimates of the trade-off between false negatives and false positives

	Estimates of false negative cases*						Estimates of false positive cases**	
	ELISA's sensitivity levels						ELISA's specificity levels	
	0.900	0.920	0.940	0.960	0.980	0.990	0.980	0.997
CP1	0.792	0.648	0.501	0.350	0.197	0.119	1.47	0.03
CP2	0.760	0.614	0.466	0.314	0.158	0.080	490.95	11.05
CP3	0.404	0.323	0.242	0.162	0.081	0.040	563.12	22.06
CP4	0.400	0.320	0.240	0.160	0.080	0.040	24547.62	3682.14
CP5	0.044	0.029	0.017	7.9E-03	2.4E-03	8.0E-04	24619.79	3693.16
CP6	0.040	0.026	0.014	6.4E-03	1.6E-03	4.0E-04	48604.29	7353.24
CP7	4.0E-04	2.6E-04	1.4E-04	6.4E-05	1.6E-05	4.0E-06	52140.62	11013.32

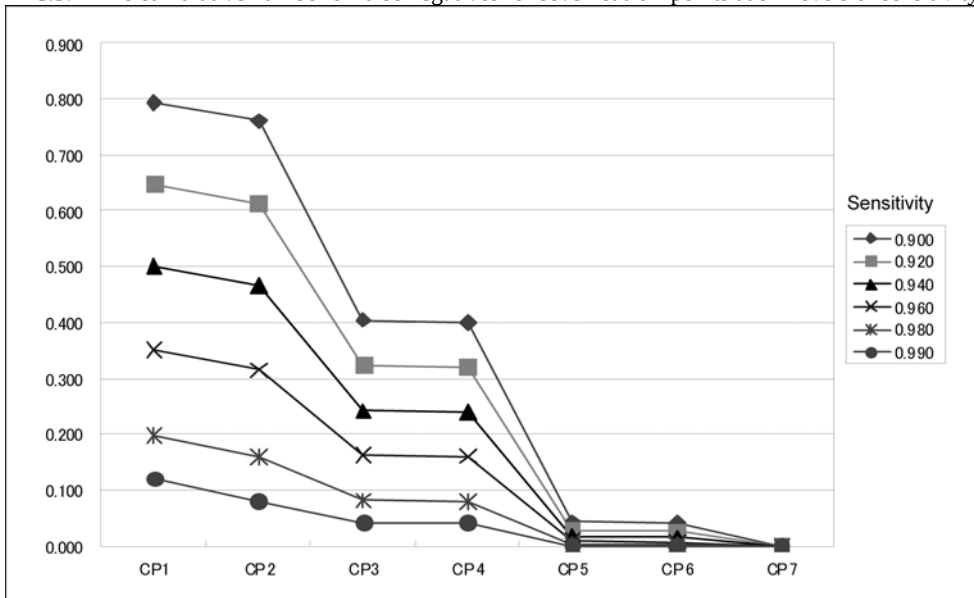
* the sensitivity levels of the secondary tests (Western blot and immuno-histochemical tests) are fixed at 99.0 percent.

** the specificity levels of the secondary tests are fixed at 99.7 percent.

According to the screening algorithm shown in Figure.1, this table displays the estimates for predicted false negatives and false positives that are to be discriminated and identified by the cut-off points at six different levels of sensitivity and two different levels of specificity in ELISA. For the simulatory conditions, the total number of cows to be screened a year is 1,227,385. Of these cattle, the annual number of BSE infected cows is four, whereas the annual number of non-BSE infected cows is 1,227,381.

FIG.2. Calculation example for BSE screening tests**FIG.2.** Calculation example for BSE screening tests

If CP1 was employed, 3.8812 cows are to be determined BSE-infected, which means that the number of false negatives, in this case, would be 0.1188 ($=4-3.8812$). If CP7 was adopted instead, the false negatives would be reduced to 4.0E-6. This example corresponds to the calculation for the estimated with 0.990 sensitivity of ELISA shown in TABLE 1.

FIG.3. The cumulative number of false negatives for seven cut-off points at six levels of sensitivity**FIG.3.** The cumulative number of false negatives for seven cut-off points at six levels of sensitivity

The graph shows the sensitivity-specific cumulative number of false negatives in ELISA for seven cut-off points, corresponding to the estimates in TABLE 1.

EVALUATION OF BSE SCREENING IN JAPAN

FIG.4. Cumulative number of false positives for seven cut-off points at two specificity levels

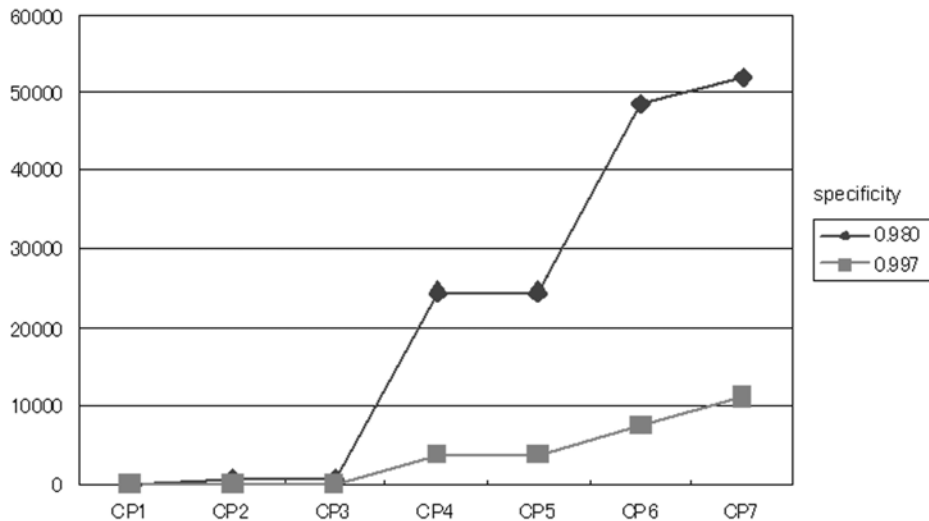


FIG.4. Cumulative number of false positives for seven cut-off points at two specificity levels
For different specificity levels, the cumulative number of false positives for BSE is shown for each of the seven cut-off points, corresponding to the estimates in TABLE 1.

Figure 3 shows the relationship between cumulative number of false negatives and sensitivity, with the condition that the cattle determined being positive based on each of the cut-off points (CP) in Figure 1 would be deterred from being in the market. It is graphically illustrated in Figure 3 that the adoption of the cut-off point CP5 (in which cows represented in the end points up to V are not to be in the market, and those in the endpoints VI, VII, and VIII are supplied in the market) can substantially reduce the occurrence of false negatives even when the sensitivity is relatively low. For instance, provided that the sensitivity of ELISA be 94.0 percent at 95 percent confidence interval (as used in 2004 European Commission's report) and that there be four infected cattle per year, there would be approximately 0.017 cases of false negative a year. The cut-off point CP4, however, at the same sensitivity level, would result in approximately 0.240 false negatives, which are about 14 times higher than those at CP5.

On the other hand, it was confirmed that the number of false positives increased substantially at CP4 as if it were a threshold. The estimates of false positives are shown in Table 1 with any possible combinations between the assumed specificity and a cut-off point. Those estimates are depicted as graphs in Figure 4. As seen in Table 1, the sensitivity of 99.7 percent for ELISA as well as the confirmatory tests, with the total number of annually screened cattle as 1,227,381, would result in 22.06 false positives, if CP3 was adopted as a cut-off point; whereas CP4 would yield 3,682.14 false positives.

DISCUSSIONS

The cut-off point CP1 is similar to that in HIV testing as adopted in Japan in its way of differentiating the infected and non-infected. The current HIV test was adopted in the 1980s, during which an attempt was made to minimize the possibility of false positives. In the 1980s, in the United States, the prevalence of AIDS and HIV rose rapidly, and it was conjectured that people's bias against those infected with AIDS and HIV necessitated a way of minimizing the possibility of false positives in order to protect people from prejudice. Nevertheless, BSE, unlike HIV, infects cattle, and from a preventive perspective emphasizing the well-being of human populations, it would be reasonable to prioritize the minimization of false negatives rather than false positives. Evidently, the current system for BSE screening does not minimize the number of false negatives.

If the primary objective is to minimize false negatives, it is the best that the cut-off point CP7 in Figure 1 is adopted in screening. In CP7, i.e., only the final point VIII in Table 1 would be non-infected and all the others (from I through VII) would be judged BSE-infected, thereby reducing the probability of false negatives. In this scheme, in other words, only cattle that have negative results on two ELISA tests, Western blot test, and immuno-histochemical testing would be forwarded to the slaughterhouse and then to the market for subsequent human consumption. Nonetheless, even though the minimization of false negatives would theoretically be ideal for consumers, the implementation of this cut-off point is practically unfeasible, considering its cost, time, and enormous amount of labor involved in it (due to the fact that majority of cattle would have both the first two ELISA tests, the Western blot and immuno-histochemical testing). Also, there could be enormous cases of false positive with the CP7, as shown in Table 1 and Figure 4.

The feasible cut-off point, in effect, would be the second best CP6. CP6, in short, treats those that have negative results in two of the ELISA tests as non-infected, and all the others would be considered infected, or not to be sent to the market. This option reduces the cost as well as labor by omitting Western blot test and immuno-histochemical testing. The financial resources originally available for these tests could be used to protect the livestock industries instead. It was confirmed in this simulation that CP6 would substantially lower the probability of false negatives, even when ELISA was held at 95.0 percent sensitivity. As a result, from a perspective of risk management, in which possibility of human errors must be taken into consideration, it can be concluded that the adoption of CP6 would be desirable, as compared to the current cut-off point.

Also, a detailed comparison of CP6, which was deemed more desirable than the current cut-off point, and the currently applied CP1 must be noted particularly with a focus on possible vCJD incidence and risk. If there is a correlation between the number of BSE infected cattle and the number of vCJD patients, it is theoretically possible to infer the number of vCJD patients in the future. The United Kingdom (UK) has had the highest number of BSE incidence. As of July 2004, 180,000 cows had been identified as BSE infected. However, one projected number of possible BSE infection was 1,000,000 even though the figure was limited for the period before the UK banned the use of meat-and-bone meal (MBM). On the other hand, there are uncertainties and unknown factors regarding vCJD (2, 8, 12). The inferred number of infected patients differs depending upon the length of the presumed latent period. Generally, a few thousand patients are predicted; Smith *et al.*, however, offered a more pessimistic figure of 5,000 (1).

Using the figure calculated by Smith *et al.* and the aforementioned projection of 1,000,000 infected cattle, the Food Safety Commission (FSC) of the Japanese Cabinet Office estimated the risk of vCJD in Japan in the report issued in September 2004 (1, 6). The

EVALUATION OF BSE SCREENING IN JAPAN

report offers two inferences with regard to the number of BSE-infected cows that have entered the food chain before October 2001: five or 35 cattle. According to the results of the FSC report, the possible vCJD incidence among 120,000,000 people in Japan would be 0.14 person (or more precisely 0.135 patient), presuming five BSE infected cows, or 0.95 person per 35 BSE infected cattle. However, if we adjust the estimates for the current Japanese population of 127,000,000, the possibility is slightly larger. Possible vCJD incidence is slightly more than 0.14 person (or more precisely 0.142 patient) per five infected cattle, whereas 1 vCJD case per 35 BSE cows. In other words, one BSE infected cow in 127,000,000 people would cause 0.03 vCJD case.

In Japan, the use of MBM has been banned since October 2001. As a result, it is expected that the possibility of BSE-prion infection will be reduced compared to the period before October 2001. If the conditions and results of this study were applied to estimate false negative cases for the period after October 2001, it would possibly result in an overestimation of the projected risk and incidence of vCJD.

Nevertheless, the fact was that the cattle infected with BSE included those born after October 2001. Hence, there is a possibility that the transmission route of BSE-prion infection may be related to factors other than the food chain. In order to assess the risk, a hypothetical situation was developed: given that all negative cases (including false negatives) would go into the food chain, how many BSE infected cows would it take to have one vCJD patient, if the cut-off point CP1 or CP6 is employed? Calculations were conducted to find out the estimates. If the sensitivity of each test is 99.0 percent, the currently used CP1 would bring about 35 BSE infected cows, provided that the total number of the screened cattle is 1,178.

If 35 infected cattle entered into the food chain, it is projected that there would be one vCJD patient. If the sensitivity of the confirmatory tests is enhanced to 99.7 percent, to have 35 false negative cases requires 1,532 infected cattle in total to be screened. On the other hand, if the cut-off point CP6 at 99.0 percent sensitivity for two ELISA tests and confirmatory tests were applied, it must get infected cattle of 350,000 needed for screening in order to have 35 false negative cases. The number needed for screening is nearly twice as many as the total number of infected cows screened in the UK up to July 2004. The aforementioned report from September 2004 (6) estimates the number of BSE infected cows after 2005-2006 as 60 at maximum. Using this figure, the potential number of vCJD incidence is 0.05 at CP1, and less than 0.0002 at CP6 in the population of 127 million. As can be seen, it was confirmed quantitatively that the risk for vCJD is very low with the current screening procedures, but it can be reduced even further.

Moreover, it was reckoned that whether or not and how the distinction of age in cattle particularly influences the risk of vCJD occurrence. Heretofore, there have been two cattle infected with BSE in Japan: one 21 months old, and the other 23 months old. From a numerical calculation, we can predict that one cow in every two years will be infected. Hypothetically, if those cattle younger than 20 months old were excluded from the screening, the aforementioned two infected cattle would still be included in the tests. Thus, there would be no impact to change the predicted risk of incidence.

Although the cut-off point CP6 has many advantages over CP1, there are also limitations. For instance, it is important to realize that using CP6 as the cut-off point and reducing false negatives would entail a higher probability of false positives shown in Figure 4, which would also be unavailable in the market. Supposing that the lower limit of specificity for both Western blot test and ELISA is 99.7 percent at 95 percent confidence interval, carrying out a simulation, with this information as well as the presumption of the number of cattle to be

tested annually as 1,227,381, resulted in a significant increase in false positives between the cut-off points CP1 and CP6: 0.03 in CP1, and 7,353 in CP6 shown in Table 1. It means, in short, there would be 7,353 non-infected cows that would be removed or eliminated before going into the market, if CP6 was used as the cut-off point. This would place a financial burden on the livestock industries. In such a scenario, consequently, administrative support for protecting the industries would probably be necessary. Nonetheless, from the perspective of public health, it is momentous to prioritize the reduction of risk for people.

Yet, to humans, the critical risk of the BSE-infected cows that “pass” the screening tests as false negatives is their potential for becoming a source of infection for vCJD. Even though there is much to be deciphered about the relationship between BSE-infected cattle and vCJD, it is considered that there is a correlation between the number of BSE-infected cows and the number of vCJD patients. Therefore, minimizing the risk of vCJD necessitates the prevention of BSE infected cows from being in the market. To achieve such ends, it is critically important to determine the cut-off points for the screening tests from the standpoint of public health or protection of people’s health above all.

As this study demonstrated quantitatively, the possibility of false negatives is low in BSE testing as practiced in Japan today. Even if the maximum number, i.e. 60, of predicted BSE infected cattle after the years 2005-2006 was applied, the estimated number of people expected to develop vCJD in the Japanese population would be 0.05 among 127 million people. It implies that the estimate is close to zero.

In conclusion, the risk analysis introduced in this study provides essential information for understanding the advantages and limitations of BSE screening in Japan, and is expected to play a significant role in constructing correct risk recognition in people.

REFERENCES

1. **Aignaux, JN, Cousens, SN, Smith, PG.** 2003. Predictability of the epidemic of variant Creutzfeldt-Jakob Disease by Back-Calculation Methods, *Stat. Meth. Med. Res.* **12**:203-220.
2. **Belay, D., Ermias, Sejvar, J., James, et al.** 2005. Variant Creutzfeldt-Jakob Disease Death - United States, *Emerging Infectious Disease* **11**(9):1351-1354. *Web information* : www.cdc.gov/eid
3. European Commission (1999): The Evaluation of Tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines.
4. European Commission (2002): The Evaluation of Tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines. (2nd study)
5. European Commission (2004): The Evaluation of 10 Rapid Post Mortem Tests for the Diagnosis of Transmissible Spongiform Encephalopathy in Bovines.
6. Food Safety Commission of the Japanese Cabinet, 2004. Nihon ni okeru BSE Taisaku ni Tsuite - Chukan Torimatome. [in Japanese]
7. **Kamae, I., Nakajo, K., Aino, H., Yanagisawa, S.** 2003. The Analysis of the Sensitivity and the Specificity of the Mass Screening System of BSE, *Rinsho Byori* **51**:1184-1187. [in Japanese]
8. **Kmietowicz, Z.** 2004. Patients informed of increased risk of vCJD contact, *British Medical Journal* **329**:702.
9. The Ministry of Agriculture, Forestry and Fisheries of Japan, 2005. Chikusan-butsu no Jyukyu. [in Japanese]
Web information: <http://www.kanbou.maff.go.jp/www/jki/mitousi/tikusan/>
10. The Ministry of Health, Labour and Welfare of Japan, 2005. BSE no Sukureeningu

EVALUATION OF BSE SCREENING IN JAPAN

ken-sa Kekka ni Tsuite (Weekly report). [in Japanese] *Web information:*
<http://www.mhlw.go.jp/houdou/0110/h1018-6.html>

11. **Onodera, T., Yoshino, T.** 1990. Recent Information in Bovine Spongiform Encephalopathy, *Nourin-suisan Gijyutsu Kenkyu Jarnal* **13**(12):10-17. [in Japanese]
12. **Pincock, S.** 2004. Government confirms second case of vCJD transmitted by blood transfusion, *British Medical Journal* **329**:251.
13. **Setbon, M., Raude, J., Fischler, C., Flahault, A.** 2005. Risk Perception of the “Mad Cow Disease” in France: Determinants and Consequences, *Risk Analysis* **25**: 813-826.