# RESEARCH REPORT - No. 0805 FY 2008-2010

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| Title of research project | Study on developing mathematical analysis methods for effective conduct and application of quantitative risk assessments |
| Research project no. | 0805 |
| Research period | FY 2008–2010 |
| Name of principal research investigator | Dr Fumiko Kasuga, National Institute of Health Science |


## Summary

The aim of the research was to develop quantitative risk assessment methods, especially probabilistic risk assessment techniques applicable to measure likelihood of foodborne illnesses.

We identified three different food - pathogen combinations and conducted microbiological risk assessments for hypothetical or actual levels of contaminations, using methods of probabilistic processing of data, uncertainty handling, sensitivity analysis and dose-response analysis. As outcome, we developed risk assessment models of: *Vibrio parahaemolyticus* infection from horse mackerel; *Campylobacter* infection from poultry; and enterohemorrhagic *Escherichia coli* (*E. coli*) infection from beef. They include respective dose-response models. We also developed relevant training materials to support risk assessors in practice of the probabilistic risk assessments.

We conducted a survey on raw meat consumption patterns and a survey on actual condition of bovine offal contaminations. Based on the survey results, we compared risks of *Campylobacter* infection in those who eat raw poultry meat and those who do not, and predicted effects of possible control measures. Regarding the risk assessment of enterohemorrhagic *E. coli* infection, we compared level of risks for 8 kinds of beef consumption patterns.

To consider practical application of the risk assessment results to risk management, we conducted a comparative survey into other industries/fields and observed ways of applying numerical target values in risk management practice. Further we carried out a survey, using the Contingency Valuation Method (CVM), to identify willingness to pay (WTP) of consumer, for the purpose of cost-utility analysis in controlling risks in foods.

Outcome of the research is provided in this report.

## Research period

2008 – 2010 (3 years)

## Introduction

It is more appropriate to take a probabilistic approach in quantitative risk assessments of hazards in foods, particularly microbiological hazards, in order to properly handle uncertainty and variability of data and accurately evaluate probability of exposure to hazards and its impact on health more accurately, and evaluate impacts of related input parameters upon results.

However, in Japan, attention and discussion are still more focused on nature and collection methods of data required for quantitative risk assessment, while research on probabilistic analysis techniques using available data and application of risk assessment results to risk management are still limited.

The purpose of this study is to improve mathematical analysis methods of data required for efficient quantitative risk assessment of food, especially probabilistic risk assessment, and to utilize obtained results in risk management.

## Purpose and methods

**1. Development of probabilistic analysis methods and dose-response analysis techniques**

As part of building specific risk assessment models, our research team searched for appropriate probabilistic risk assessment models, particularly dose-response models, and developed methods for assessment and data analysis.

**2. Analysis of techniques for semi-quantitative and qualitative risk assessments**

**A: Qualitative and quantitative risk assessments of *Campylobacter* cross-contamination during poultry meat preparation**

In the whole range of farm-to-fork type probabilistic risk assessment model, we focused on cross-contamination of *Campylobacter* to RTE (ready-to-eat) food during poultry meat preparation at homes and in restaurants. Using deterministic models, we investigated relationship between prevalence and contamination level of retail poultry meat and the average number of *Campylobacter* and contamination probability in RTE food, as well as influence of various food preparation habits on the average number of *Campylobacter* and contamination probability in RTE food, and on the probability of *Campylobacter* infection.

**B: Beef consumption survey for risk assessment of enterohemorrhagic E. coli**

The main infection sources of enterohemorrhagic *E. coli* (EHEC) are assumed to be raw liver, liver *sashimi and* other raw meat. In the survey, we asked frequency of consumption of raw and cooked beef and bovine offal in barbecue restaurants (including Korean barbecue and *horumon-yaki* or grilled offal to obtain information for EHEC risk assessment.

**C: Consideration of beef contamination survey methods for risk assessment of enterohemorrhagic E. coli**

There exist survey data on enterohemorrhagic *E. coli* contamination of beef at retailers; however, very little, if any, data are available on contamination of beef, particularly offal meat served at restaurants. Thus we

considered survey methods, and conducted a trial survey.

**3. Development of uncertainty handling techniques and analysis of qualitative risk assessment techniques**

We built a simulation model for cross-contamination of Campylobacter at the individual carcass level during poultry processing, which is an important control point of poultry contamination, and considered quantitative risk assessment taking account of uncertainty. In addition, we conducted a questionnaire survey on raw meat consumption by region, examined its relationship with the number of food poisoning cases of *E.coli* O157 and other pathogens, and carried out qualitative and epidemiological analysis.

**4. Consideration on applicability of quantitative risk assessment to setting microbiological metrics of food products**

**D: Application of quantitative risk assessment to setting microbiological metrics of food products**

International discussions have been held on how risk assessments should be utilized for establishing Food Safety Objectives, Performance Objectives, Performance Criteria and other microbiological metrics of food products. This study attempts to apply quantitative risk assessment results with reference to thosein industries and fields other than food hygiene; particularly, we reviewed literature on these metrics, collected cases which examine a relationship between performance objectives value set for risk management and results of quantitative risk assessment used in industries and fields other than food hygiene, and considered possibilities of their application to microbiological risk assessment.

In addition, we used sample models to examine methods for scenario analysis of uncertain parameters through back-calculation from simulated results of quantitative risk assessment (Value at Risk, etc.), and considered possibilities for their application to microbiological risk assessment.

**E:Cost-utility analysis of quantitative risks**

In order to contribute to practical application of quantitative risk assessment, we examined willingness to pay to avoid infection with enterohemorrhagic *E. coli*, *Campylobacter* and norovirus.

**5. Development of probabilistic analysis methods and sensitivity analysis techniques**

**F: Development of risk assessment models for Campylobacter contamination in poultry meat, and of methods to** evaluate effects of preventive measures against food poisoning

Based on actual conditions of poultry production and slaughtering as well as distribution, retail, preparation and consumption of poultry meat (especially, raw meat) in Japan, we developed a probabilistic risk assessment model of *Campylobacter* contamination from a poultry farm to a dining table (farm-to-fork).

**G: Improvement of risk assessment model 1**

We presented the outline of the risk assessment model developed in F as well as the current *Campylobacter* infection risks and evaluation of the effects of preventive measures against food poisoning at domestic and international academic societies and conferences involved in probabilistic risk assessment of food products. The model was improved by peer reviews of many experts both in Japan and abroad.

**H: Improvement of risk assessment model 2**

The model formulated theoretically in G for predicting change in contamination level during the chilling process was improved by implementing through reality check to enable a more accurate evaluation of impacts of reduced on-farm contamination rate as well as impacts of lowered on-farm contamination rate combined with strategic processing. Besides, the effects of various preventive measures against food poisoning were evaluated using sensitivity analysis.

**I: Development of risk assessment model for enterohemorrhagic E. coli infection from beef**

We investigated the risk of O157 infection from beef by collecting data such as contamination rates of beef at retailers and implementing a probabilistic model to evaluate likelihood of developing the disease by different beef consumption patterns as an index.

**6. Development of methods for conducting quantitative risk assessment and its application (summary)**

**J: Survey on enterohemorrhagic E. coli contamination in bovine offal and its level**

We purchased bovine offal at retailers via Internet shopping, and sent it to the test laboratory for qualitative EHEC detection. In addition, we carried out semi-quantitative tests and calculated detection limit at the National Institute of Health Sciences to collect basic data for risk assessment of EHEC infection from beef.

## Research results – FY 2008

### Summary

We created a probabilistic risk assessment model for *Campylobacter* infection from poultry meat, based on all data and information obtained from: field surveys of poultry farms and slaughterhouses; analysis of literature data; and previous year's surveys by the Food Safety Commission.

We estimated risks based on the present number of people infected, and compared estimated risk reduction effects of assumed risk management measures, namely (i) reduction of *Campylobacter* contamination rate by sanitary control at farms, (ii) time-separated processing of contaminated and non-contaminated poultry at slaughterhouses, (iii) provision of required chlorine level in chilling water at poultry slaughterhouses, (iv) reduction of raw poultry consumption, (v) reduction of insufficient cooking, (vi) prevention of cross-contamination during food preparation.

The analysis approach, model configuration and risk assessment results were directly used in the risk assessment documents prepared by the working group of Expert Committee on Microorganisms and Viruses, Food Safety Commission. Accordingly, the result of the present research was reflected in the Risk Assessment Report.

### Research results

**1. Risk assessment of *Campylobacter* infection from poultry meat**

**1-1. Overall risk assessment**

Based on field surveys of poultry farms and slaughterhouses, analysis of literature data, results of previous

year's surveys by the Food Safety Commission as well as discussions held in the research group meetings, we created a probabilistic risk assessment model for *Campylobacter* infection from poultry meat. We estimated risks to be presented as number of those infected, and compared estimated risk reduction effects of possible control measures, namely (i) reduction of *Campylobacter* contamination rate by sanitary control at farms, (ii) strategic slaughtering: time-separated processing of contaminated and non-contaminated poultry at slaughterhouses, (iii) provision of required chlorine level in chilling water at poultry slaughterhouses, (iv) reduction of raw poultry consumption, (v) reduction of insufficient cooking, (vi) prevention of cross-contamination during food preparation.

According to the research results, the mean number of those infected is 100,007,271 persons per year; this figure, however, is strongly affected by the large number of infected people with very small probability. This is equivalent to 1.35 cases per year. Among them 0.95 occurred at home and 0.40 at restaurants. The number of poultry meat consumptions is 164 times per year at home, and 41 times at restaurants. The frequency of poultry consumption at home is 4 times higher; however, the mean number of incidence at home is just 2.4 times more often than incidence at restaurants. This means that infection risk is higher at restaurants.

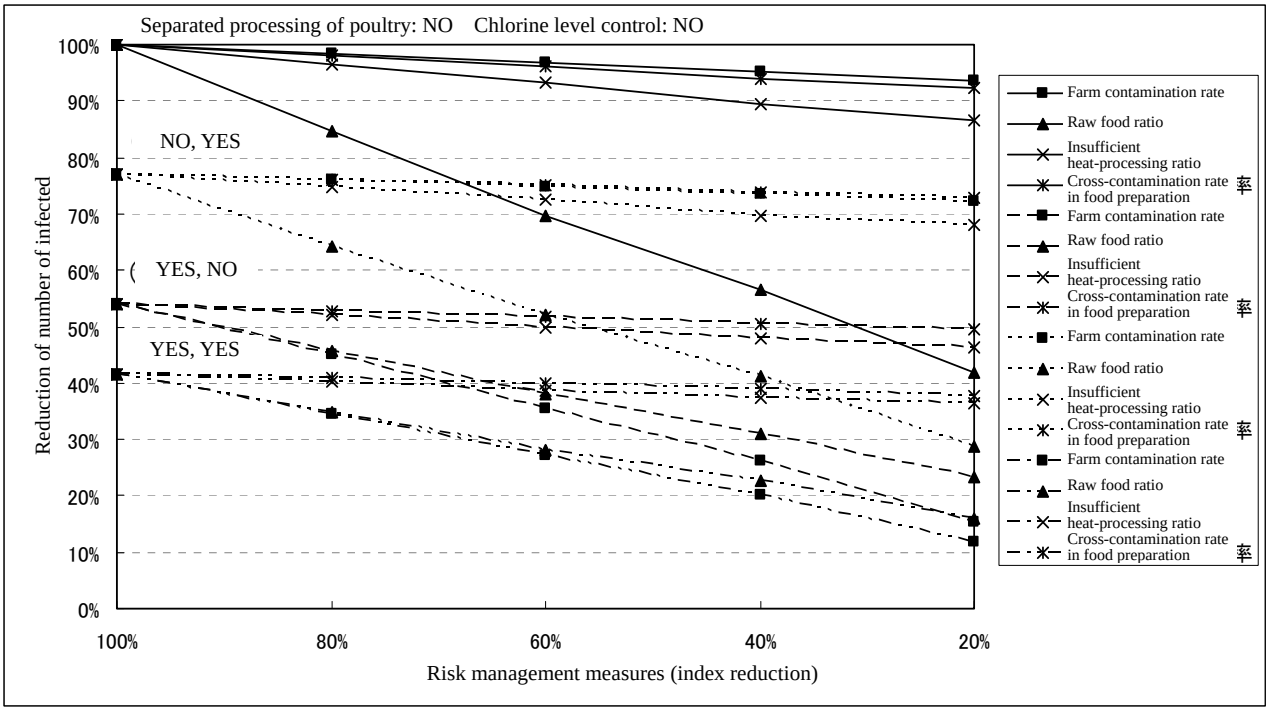



Fig. 1. Estimated effects of assumed risk management measures

Fig. 1 illustrates the effects of preventive measures against food poisoning in terms of how food poisoning risk decreases with reduction of respective index (e.g., contamination rate at farm). The most effective measure against food poisoning is strategic processing at poultry slaughterhouse. When the strategic processing is not practiced, the second most effective measure is reduction of raw food consumption, for example, by means of consumer education. Reduction of contamination rate at farm has a limited effect when implemented alone, because its effect is partially canceled out by cross-contamination at the slaughterhouse. However, reduction of contamination rate at farm becomes the most effective measure provided that separated processing is also

practiced at slaughterhouses. Thus, reduction of on-farm contamination rate was suggested to have a sufficient impact only if cross-contamination is avoided at slaughterhouses.

### 1-2. Simulation model of Campylobacter cross-contamination during poultry processing

We created a cross-contamination model for spread of contamination within a lot of slaughtered poultry. We assumed poultry slaughtering process as “decontamination” and “cross-contamination” for simplicity. We varied contamination rate of pre-slaughtered lot in the range of 0-100% using 10% increments; the mean number of Campylobacter in a contaminated poultry was set to values taken from previous studies (log104.2, log105.5, log107.8). *Campylobacter* was assumed to be eliminated through defeathering and evisceration, and the success probability was set (0.3, 0.6, 0.9). As for the process of cross-contamination, we assumed that a contaminated carcass contaminates the following one, and that cross-contamination takes place when the number of transferred bacteria exceeds certain value, using formula for estimating rate of transmission. The change in contamination rate in post-slaughtered lot (Fig. 2) suggested that contamination spreads to a whole lot through cross-contamination if the number of bacteria in contaminated poultry is high, even though contamination rate of the pre-slaughtered lot was low.

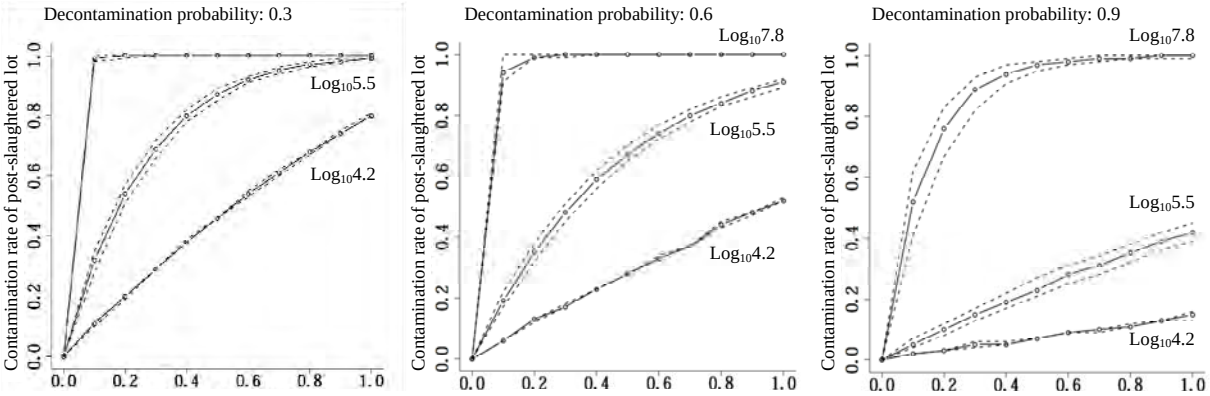



Fig. 2. Average bacterial count of contaminated poultry and change in contamination rate in post-slaughtered lot. Numbers in the graphs indicate average bacterial count of contaminated poultry (CFU). The dashed lines indicate 95% confidence interval.

### 1-3. Detailed investigation of cross-contamination during food preparation

Regarding the stage of food preparation, we assumed cross-contamination mediated by chopping boards and fingers, and created a model to simulate the number of *Campylobacter* transferred from raw poultry meat to RTE food products using cross-contamination probability depending on food preparation habits (preparation sequence of raw poultry meat and RTE food, use of separate chopping boards, washing or not washing of chopping boards and hands, etc.), bacterial survival rates depending on the washing methods of chopping boards and fingers, and bacterial transfer rate in cross-contamination (cross-contamination rate). We used the result of nationwide questionnaire survey conducted by the Food Safety Commission on food preparation habits, bacteria survival rates depending on the washing methods in domestic previous studies, and cross-contamination rates in foreign previous studies.

### 1-4. Analysis of dose-response relationship

We conducted a comparative study of models used for risk assessment in foreign countries. Data commonly used for the models are taken from ingestion experiments with two kinds of strains, carried out by Black et al. with healthy adult volunteers. The procedure currently considered as standard is to use parameters of beta-Poisson model (approximate form), obtained by Medema et al. from average intake and average infection rate, to figure out probability of infection by one strain by beta distribution, then to calculate infection rate at a given number of actually ingested Campylobacter (Conditional Dose-Response Relationship) by a binomial probability, to estimate proportion of disease in response to infection using the mentioned data by Black et al., and finally to determine the proportion of those who develop disease among those who are infected. The dose-response relationship proposed by Teunis et al. using actual data on milk poisoning during farm visits is also used for some risk assessments for comparison. It is pointed out that the approximate form is not sufficient for evaluation of uncertainties of dose-response relationship models (Teunis et al.). Thus we continue reviewing related papers and the latest assessments.

Besides, assuming that involvement of immune mechanisms in *Campylobacter* infection will be reflected in future models, we reviewed bibliographical information in this field.

**2. Application of quantitative risk assessment to setting microbiological metrics of food products**

When considering how to apply results of microbiological risk assessment to risk management, in this financial year we first studied literature on metrics such as Food Safety Objective, Performance Objective and Performance Criteria. Besides, we collected and compared examples of performance objectives and their relation to results of quantitative risk assessment in fields other than food hygiene (banking, etc.), and considered applicability to microbiological risk assessment. In addition, we considered DALYs, a method to convert health damages of various severity into years of healthy life lost due to disability, and conducted a pilot questionnaire survey about apprehension of the underlying concept of years lost due to disability.

**Discussion and conclusions**

A model for probabilistic risk assessment of *Campylobacter* infection from poultry meat was created as a result of discussion and cooperative work of the entire research team. We estimated risks based on the present number of infected, and compared and evaluated risk reduction effects of possible risk management measures, namely (i) reduction of *Campylobacter* contamination rate by sanitary control at farms, (ii) time-separated processing of contaminated and non-contaminated poultry at slaughterhouses, (iii) provision of required chlorine level in chilling water at poultry slaughterhouses, (iv) reduction of raw poultry consumption, (v) reduction of insufficient cooking, and (vi) prevention of cross-contamination during food preparation.

The analytical approach, model configuration and risk assessment results are directly cited in the risk assessment documents made by the working group of Expert Committee on Microorganisms and Viruses, Food Safety Commission. The results of the present research are reflected simultaneously in the Risk Assessment Report. Thus we presented a model for probabilistic risk assessment procedure with respect to food-borne microorganisms.

**Research results – FY 2009**

## Summary

The risk assessment of *Campylobacter* infection from poultry meat performed in FY 2008 was directly reflected in risk assessment report by the Expert Committee on Microorganisms and Viruses, and was published by the Food Safety Commission in June 2009. However, the risk assessment model was modified in response to comments obtained during presentations in international conferences etc. so as to develop mathematical analysis methods for application to future quantitative risk assessment.

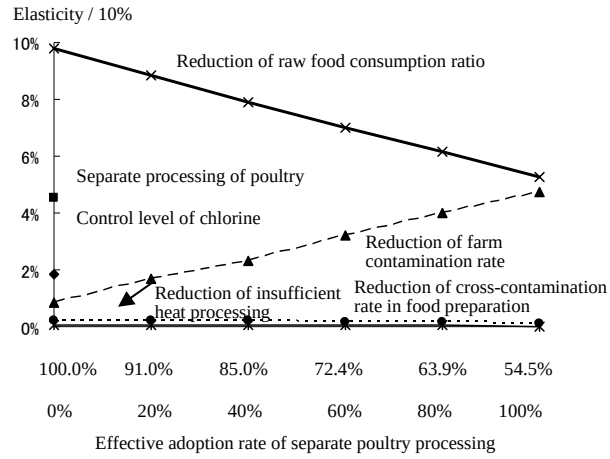
In addition, we proposed an outline of possible risk assessment of enterohemorrhagic *E. coli* in beef, which was the subsequent target of risk assessment by the Expert Committee on Microorganisms and Viruses.

For that purpose, we collected and studied literature on dose-response relationship, created a simulation model of *Campylobacter* infection from poultry meat in slaughtering process, conducted a survey on consumption of raw beef and bovine offal at barbecue restaurants, and also considered applying quantitative risk assessment to setting microbiological metrics of food products.

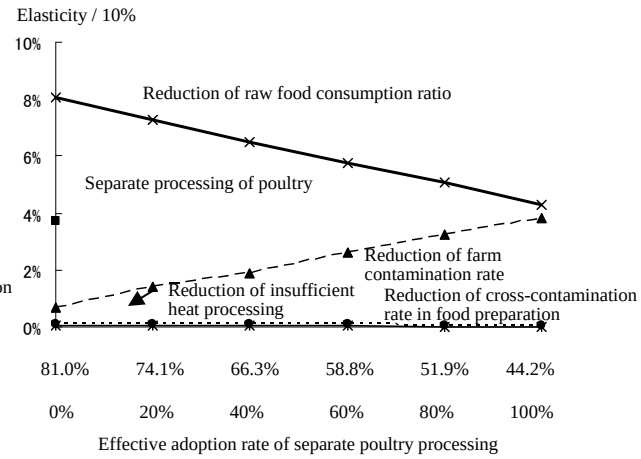
## Research results

### 1. Modification of *Campylobacter* risk assessment model

In the process of modification of the model which takes account of effective adoption rate of strategic processing of poultry, we found that the result of sensitivity analysis, performed to assess the effects of various measures against food poisoning with 50,000 simulation runs and 204 scenarios, was almost linear. We presented results of the sensitivity analysis, separately for those who eat raw food and those who do not, using elasticity, which shows how infection risk decreased with annual infection risk as baseline when the index of each measure against food poisoning was changed by 10% (the diagrams below).

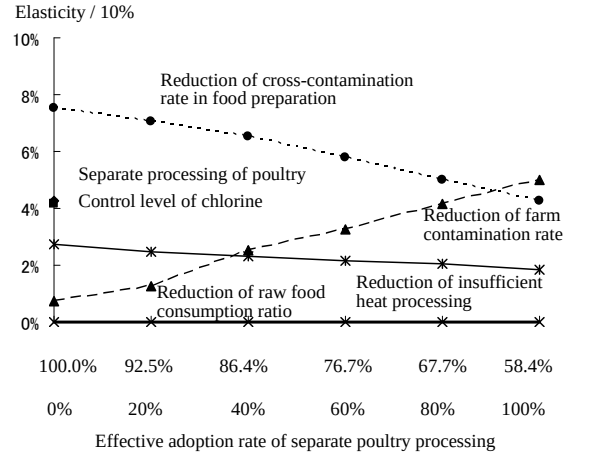



Effective adoption rate of separate poultry processing and elasticity of food poisoning countermeasures for habitual raw food eaters (without chlorine level control)

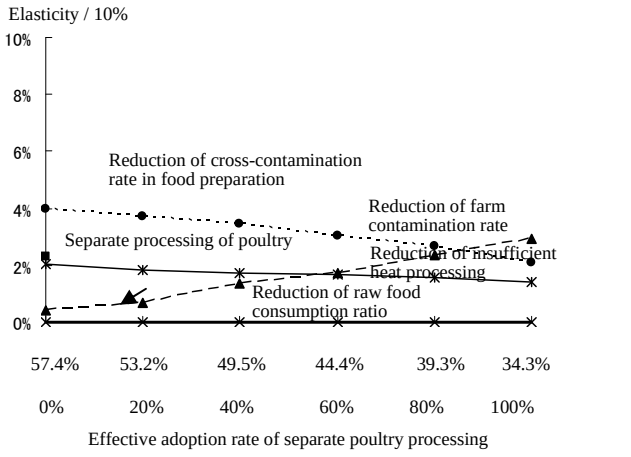



Effective adoption rate of separate poultry processing and elasticity of food poisoning countermeasures for habitual raw food eaters (with chlorine level control)

* The figures below the axis of effective adoption rate of strategic processing show reduction of infection risk with the annual infection risk as baseline for respective effective adoption rate.




Effective adoption rate of separate poultry processing and elasticity of food poisoning countermeasures for habitual raw food non-eaters (without chlorine level control)




Effective adoption rate of separate poultry processing and elasticity of food poisoning countermeasures for habitual raw food non-eaters (with chlorine level control)

* The figures below the axis of effective adoption rate of strategic processing show reduction of infection risk with the annual infection risk as baseline for respective effective adoption rate.

A study using the model by RIVM (2005) showed that the change of contamination level of poultry in the process of chilling at a slaughterhouse could be modeled as described below.

The constant *a* refers to release probability of Campylobacter per cfu in contaminated poultry, constant *c* refers to probability of inactivation/decontamination, and constant *b* refers to probability of adhesion of Campylobacter to contaminated poultry per cfu in the chilling water. Besides, assume that the same number of poultry always flows through the water tank; *N* refers to the number of poultry flowing through the water tank at a certain point of time, *M* (g) refers to the average weight of one carcass, *m* (CFU/g) refers to the level of poultry contamination immediately before chilling, *W* ($m^3$) refers to the volume of water tank, and *w* (CFU/$m^3$) refers to contamination level of chilling water.

Let $m'$ be the level of poultry contamination immediately after chilling, then the total number of Campylobacter in $N$ poultry carcasses is expressed as $NMm' = NMm(1 - a)(1 - c) + Wwb$, which means $m' = m(1 - a)(1 - c) + Wwb/NM$.

The values of RIVM (2005) can be used for $a$, $b$, and $c$. The value for $N$ and $W$ can be given based on the data of chilling water tank of typical poultry slaughterhouses. $M$ has been already set to 3 kg (big broiler) in the model. Finally, regarding contamination level of chilling water, $w$, assuming it as a function of the poultry contamination rate only, and using data on the contamination level of chilling water $w$ in case of handling highly contaminated poultry (contamination rate $v$), the relation between the contamination level of chilling water and poultry contamination rate can be expressed as a model using the straight line $w = s \cdot v$ ($s$: coefficient of proportionality) through the origin.

In this study, $m'$ is assumed equal to the level of poultry contamination at the stage of distribution and retail; hence data on contamination level at the stage of distribution and retail can be taken from previous studies. Therefore, $m$ can be back-calculated by specifying the mentioned values, and the change rate of contamination level $m'/m$ can be obtained.

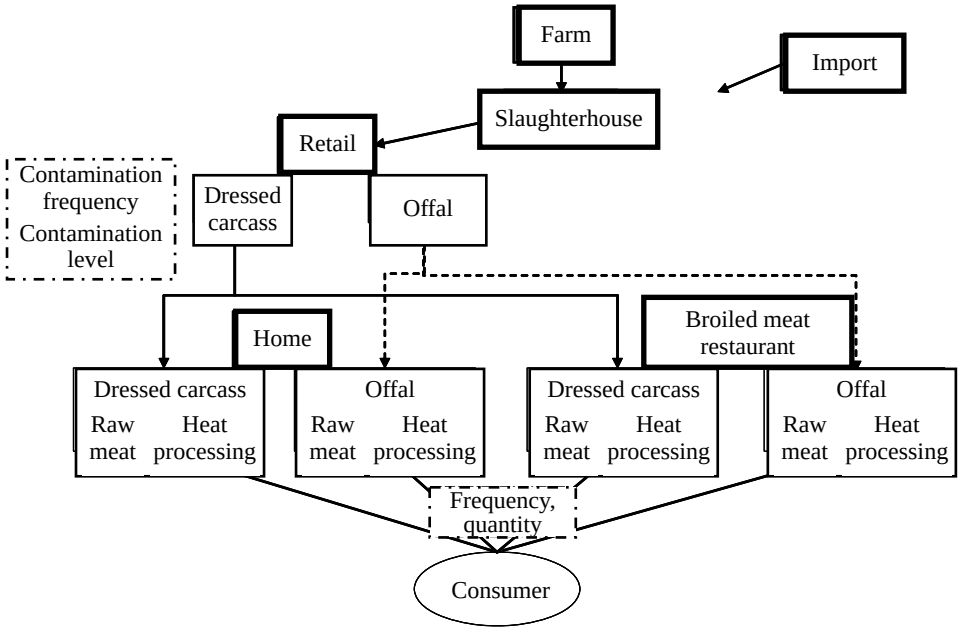
## 2. Proposed outline of risk assessment for enterohemorrhagic *E. coli* contamination in beef

As pointed out by the study group of Expert Committee on Microorganisms and Viruses, Food Safety Commission, it is presently difficult to collect information and data on distribution of bovine offal. Thus we concluded that it is difficult to carry out exposure assessment along the food chain, from production to consumption (shown by downward arrows in the diagram below).

On the other hand, proportion of different types of beef actually consumed by consumers can be estimated directly by analyzing consumption pattern of consumers (shown by upward arrows in the diagram below). In this financial year, a questionnaire survey about beef consumption at broiler meat restaurants were analyzed in sub-study by Yamamoto; besides, frequency of beef consumption at home and at restaurants as well as frequency of raw beef consumption at home were surveyed by the Food Safety Commission in FY 2006. An overall picture of beef consumption patterns can be drawn by combining these survey results.

In addition, exposure by types of beef (offal, dressed carcass) and by processing method (with or without cooking) can be assessed to some extent through a combination of contamination rate and contamination level of enterohemorrhagic *E. coli* in bovine offal and dressed carcass in the final stages of distribution (retail sales immediately before purchased by restaurants). Since the available data on contamination rate and contamination level in bovine offal at the distribution stage was very limited, our research team decided to conduct an independent survey in the following year.

Thus, targeting sporadic cases, that actually make up the majority of patients, risk assessment to estimate contribution rate to enterohemorrhagic *E. coli* infection by types of beef and different consumption patterns was supposed to be feasible. .




Schematic diagram of beef consumption

**Discussion and conclusions**

Regarding *Campylobacter* risk assessment, a more realistic risk assessment model was developed taking account of impacts of cross-contamination rate and adoption rate of strategic processing.

As for risk assessment of enterohemorrhagic *E. coli*, the research team agreed that approaching consumers was more efficient, and thus we started with developing a qualitative framework of the model, followed by a questionnaire survey as the first step of qualitative assessment. We believe that this survey produced valuable baseline qualitative and quantitative data on consumption patterns of meat, particularly raw meat, at broiled meat restaurants,, which is essential for EHEC risk assessment. In addition to such risk assessment, epidemiological analysis by other research teams is revealing population proportional attributable risk from risk factors of sporadic cases of enterohemorrhagic *E. coli* O157. Further improved accuracy of estimation is expected by putting all the information together.

We also considered efficient application of results of quantitative risk assessment to risk management. Scenario analysis with parameters back-calculated from simulated results of quantitative risk assessment is efficient to understand what value of which parameter results in exceeding microbiological metrics (maximum values). Identification of such parameters enables deliberation of measures to reduce probability of reaching microbiological metrics. Although we used only sample models in this financial year, we are going to verify our results using actual quantitative models of microbiological risk assessment. In addition, economic aspects of public health concerns are supposed to be considered to understand a health hazard index in risk management; however, the concept of citizens' willingness to pay has hardly been introduced. This concept is expected to become a new index.

**Research results – FY 2010**

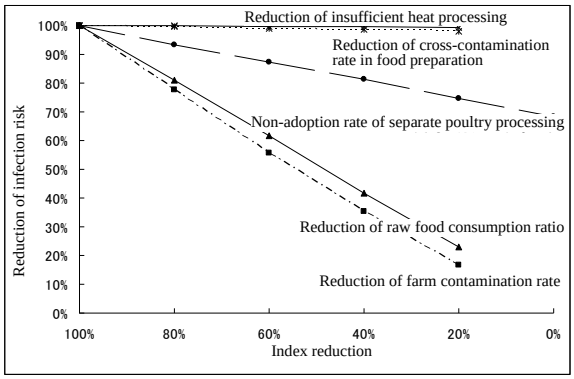
## 1. Modification of *Campylobacter* risk assessment model

### 1-1. Probability of infection per serving for those who eat raw food and those who do not

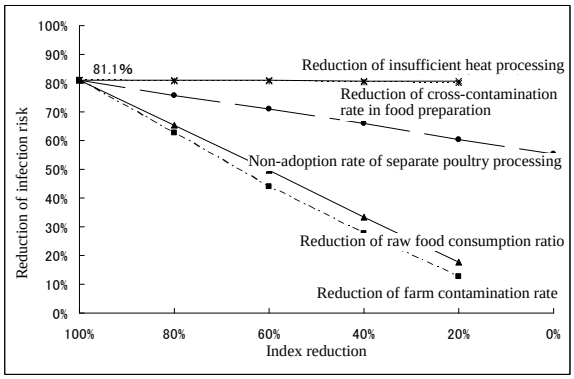
We improved the risk assessment model by introducing the change in contamination level at a slaughterhouse, divided incompleteness of farm surveys from adoption rate of strategic poultry processing, and the cross-contamination rate at a slaughterhouse. Probability of infection per serving of poultry dish was calculated for the base case for those who eat raw food and those who do not, which was close to the results obtained with the model developed by FY 2009.

### 1-2. Effects of preventive measures against food poisoning upon reduction of infection risk

Such measures as reduction of insufficient cooking or prevention of cross-contamination during food preparation had little effect among those who eat raw food, just as the previous model suggested. On the other hand, while the previous model suggested dramatic reduction of infection risk by reduced rate of raw food consumption, the modified model showed that the risk reduction by reduced on-farm contamination rate had a greater effect.

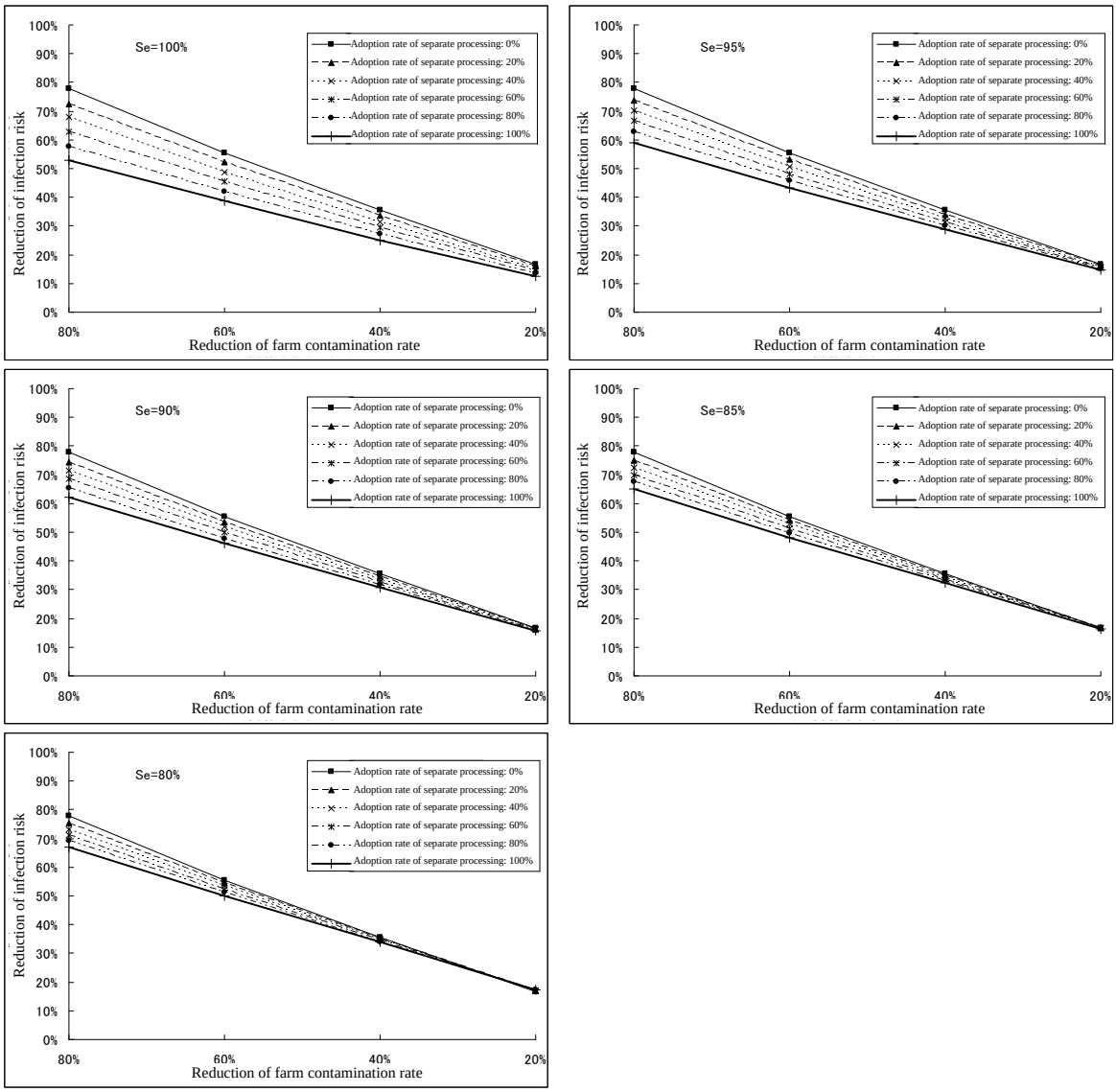



Efficiency of food poisoning countermeasures for infection risk reduction for habitual raw food eaters (without chlorine level control)




Efficiency of food poisoning countermeasures for infection risk reduction for habitual raw food eaters (with chlorine level control)

With 100% sensitivity, lowered on-farm contamination combined with strategic processing reduces infection risk in the following way. When on-farm contamination rate is reduced to 80%, infection risk decreases by about 2.5% per 10% of adoption rate of strategic processing (infection risk drops by about 25% from 77.8% to 52.9% as the adoption rate is changed from zero to 100%). On the other hand, when on-farm contamination rate is reduced to 20%, risk is reduced by one-sixth, that is, 0.4% (infection risk is reduced by about 4% from 16.8% to 12.6% as the adoption rate is changed from zero to 100%). The effect of strategic processing was reduced with lower test sensitivity due to cross-contamination caused by infected poultry with a false negative test result.




Efficiency of farm contamination control combined with separate poultry processing (with regard to test sensitivity) for infection risk reduction in case of habitual raw food eaters

Effects of on-farm contamination control combined with strategic processing upon reduction of infection risk for those who eat raw food (sensitivity is considered)

As for those who do not eat raw food, reduction of raw food consumption rate has no impact on risk reduction, while reduction of insufficient cooking exerts a certain effect, as suggested by the previous model. On the other hand, while the previous model suggested that reduced cross-contamination rate during food preparation was more effective in reducing infection risk compared to other preventive measures against food poisoning, the modified model showed that reduced on-farm contamination was more effective. More precisely, the risk was reduced by 8 to 14% per 10% of reduction of on-farm contamination rate, and by roughly 8% per 10% of reduction of cross-contamination rate during food preparation. In addition, strategic poultry processing also had a certain effect, namely, the risk was reduced by 2 to 3% per 10% of adoption rate.

The trends in risk reduction for those who do not eat raw food basically correspond to the trends for those who eat raw food. With 100% sensitivity, reduction of on-farm contamination rate combined with adoption

of strategic processing reduces infection risk in the following way. When on-farm contamination rate is reduced to 80%, infection risk decreases by about 2% per 10% of adoption rate of strategic processing (infection risk is reduced by about 19% from 71.7% to 52.6% as the adoption rate is changed from zero to 100%). On the other hand, when on-farm contamination rate is reduced to 20%, risk is reduced by one-tenth, that is, 0.2% (infection risk is reduced by about 2.4% from 10.2% to 7.8% as the adoption rate is changed from zero to 100%).

Just as with those who eat raw food, the effect of strategic processing was reduced with lower test sensitivity.

Outline of procurement of bovine offal samples

| | |
|---|---|
| Number of suppliers | 10 |
| Number of goods | 52 |
| Number of providers | 12 |
| Number of inspection agencies | 2 |
| Number of procured samples | 180 |
| Period of procurement and inspection | 4 months |

## 2. Development of risk assessment model for Enterohemorrhagic *Escherichia coli* infection from beef

### 2-1. Survey of enterohemorrhagic *E. coli* contamination in bovine offal and its level

Raw meat is considered as the main infection source of Enterohemorrhagic *E. coli* (EHEC), and data collection on EHEC contamination is essential to conduct risk assessment. In Japan, EHEC contamination in dressed carcass is surveyed every year, and the results are published; however, available data on EHEC contamination in offal meat is very limited, and more detailed data is necessary.

**Purpose**

The purpose of the survey is to describe EHEC contamination status (prevalence and contamination level) of bovine offal at retailers in order to assess the risk of EHEC from beef.

**Contents and methods**

We purchased bovine offal via Internet shopping, and sent it to the test laboratory for qualitative EHEC detection. In addition, we carried out semi-quantitative tests at the National Institute of Health Sciences to collect basic data for risk assessment of EHEC infection from beef.

***Qualitative tests***

We purchased a total of 180 samples of chilled domestic bovine offal via the Internet, and sent 80 of them to the Japan Food Research Laboratories and 100 to the Japan Frozen Food Inspection Corporation, and 52 of them to the Hyogo Prefectural Institute of Public Health and Consumer Sciences via a chilled delivery service for qualitative tests and isolation of EHEC..

The qualitative tests were conducted in the following way. A 25 g portion of each sample was diluted with 225 ml buffered peptone water (BPW) in a Stomacher bag,, and then homogenized for 1 minute. After incubation for 18 hours at 42°C, a 1 ml aliquot of the enrichment broth was centrifuged for 5 minutes at 15,000 rpm, and then the cell pellets were re-suspended in 100 µl PrepMan Ultra solution. After boiling and centrifuging, the supernatant was used for PCR. *Shiga* toxin genes (*stx*) were detected by PCR, and the *stx* -positive samples were further subjected to PCR to detect O157, O26 and O111.

The samples that were PCR positive for any of O157, O26 or O111, were subject to bacterial isolation. One ml of the broth was mixed with 50 μl of DynaBeads specific for O157, O26 or O111, and the target serotype bacteria was enriched according to the manufacturer's instructions. The enriched broth was stained in KBM STEC Chrom agar and CT-SMAC for O157, in KBM STEC Chrom agar and CT-RMAC for O26, and in KBM STEC Chrom agar and CT-SBMAC for O111, respectively, and then incubated for 18 to 20 hours at 37°C. Suspicious colonies were extracted on trypticase soy agar, and cultured overnight at 37°C. The serotype was identified using O-group antisera (anti-O157, O26, and O111). In addition, genetic typing of *Shiga* toxin genes of isolated strains was performed by PCR.

***Semiquantitative test***

A total of 37 bovine offal samples for semi-quantitative tests were purchased via the Internet and at retail shops. Three 30-g pieces of each sample were diluted in 270 ml of BPW in filtered Stomacker bags, and homogenized for 1 minute. Then 10-fold serial dilutions were prepared by adding 25 ml of each sample solution to 225 ml of BPW. Similarly 100-fold serial dilutions were prepared by adding 2.5 ml of each sample solution to 247.5 ml of BPW. After incubation for 20 hours at 42°C, 1 ml of the broth is centrifuged for 1 minute at 15,000 rpm, and then the cell pellets were re-suspended in 50 μl PrepMan Ultra solution. After boiling for 10 minutes at 95°C and centrifuging for 2 minutes at 15,000 rpm, the supernatant was used for PCR. *Shiga* toxin genes (*stx*) were detected by PCR, and the MPN method in three stages was used for semiquantification. The *stx*-positive samples were further subjected to PCR to detect O157, O26 and O111.

***Calculation of detection limit***

We calculated detection limits of PCR assay for Shiga toxin genes. Four O157 strains were cultured overnight at 37°C in BPW; then serial dilutions were prepared, and inoculated by 100 μl per 5 g of bovine offal. After adding 45 ml of BPW and culturing in the same way as with qualitative and semi-quantitative tests, *Shiga* toxin genes were detected by PCR. At the same time, 100 μl of serial dilutions used for inoculation were stained in trypticase soy agar, and cultured overnight at 37°C; after that, the number of inoculum of bacteria was calculated by counting colonies.

**Results**

***Qualitative test results***

Data on 180 samples analyzed at the Japan Food Research Laboratories and Japan Frozen Food Inspection Corporation are summarized, except for 1 large-intestine sample imported from Mexico. Twenty samples (11.2%) out of 179 were *stx*-positive. PCR test results by body part are shown in Table 1. One sample of heart (33.3%), 3 samples of liver (10.0%), 4 samples of rumen (21.1%), 2 samples of reticulum (11.8%), 1 sample of omasum (3.4%), 2 samples of abomasum (10.5%), and 7 samples of small intestine (17.1%) were *stx*-positive; no samples of large intestine were found *stx*-positive. Among the 20 *stx*-positive samples, 4 were positive for only O157, and 1 was positive for both O157 and O26, while none were found positive for O111. By body part, *stx*-positive for O157 only was found in one sample in liver, one in rumen, and two in small intestine; the sample positive for both O157 and O26 was in

omasum.

PCR test results by retail shop (prefecture) are shown in Table 2. None were found *stx*-positive among samples in Akita prefecture, while 1 out of 2 samples in Tokyo, 1 out of 26 samples (3.8%) in Shiga, 8 out of 27 samples (29.6%) in Nara, 4 out of 30 samples (13.3%) in Hyogo, 1 out of 34 samples (2.9%) in Yamaguchi, 1 out of 15 samples (6.7%) in Tokushima, 2 out of 20 samples (10.0%) in Fukuoka, and 2 out of 20 samples (10.0%) in Saga were found *stx*-positive.

The number of isolated strains was O157 strains from three samples and O26 strain from one sample. One out of three O157 strains isolated was *stx*-negative, and was therefore excluded (Table 1, Table 2). Thus, EHEC O157 was isolated from 2 out of 179 samples (1.1%), and EHEC O26 from 1 sample (0.56%).

Table 1. Results for detection of enterohemorrhagic *E. coli* in offal (by body part)

| Body part | Number of samples | Number of PCR positive | | | | Number isolated | Serotype |
|---|---|---|---|---|---|---|---|
| | | *stx* | O157 | O26 | O111 | | |
| Heart | 3 | 1 | 0 | 0 | 0 | 0 | |
| Liver | 30 | 3 | 1 | 0 | 0 | 0 | |
| Rumen | 19 | 4 | 1 | 0 | 0 | 0 | |
| Reticulum | 17 | 2 | 0 | 0 | 0 | 0 | |
| Omasum | 29 | 1 | 1 | 1 | 0 | 1 | O26 |
| Abomasum | 19 | 2 | 0 | 0 | 0 | 0 | |
| Small intestine | 41 | 7 | 2 | 0 | 0 | 2 | O157 |
| Large intestine | 15 | 0 | 0 | 0 | 0 | 0 | |
| Others | 6 | 0 | 0 | 0 | 0 | 0 | |
| Total | 179 | 20 | 5 | 1 | 0 | 3 | |

Table 2. Results for detection of enterohemorrhagic *E. coli* in offal (by shop)

| Retail shop (prefecture) | Number of samples | Number of PCR positive | | | | Number isolated | Serotype |
|---|---|---|---|---|---|---|---|
| | | *stx* | O157 | O26 | O111 | | |
| Akita | 5 | 0 | 0 | 0 | 0 | 0 | |
| Tokyo | 2 | 1 | 0 | 0 | 0 | 0 | |
| Shiga | 26 | 1 | 0 | 0 | 0 | 0 | |
| Nara | 27 | 8 | 3 | 1 | 0 | 2 | O26, O157 |
| Hyogo | 30 | 4 | 1 | 0 | 0 | 1 | O157 |
| Yamaguchi | 34 | 1 | 0 | 0 | 0 | 0 | |
| Tokushima | 15 | 1 | 1 | 0 | 0 | 0 | |
| Fukuoka | 20 | 2 | 0 | 0 | 0 | 0 | |
| Saga | 20 | 2 | 0 | 0 | 0 | 0 | |
| Total | 179 | 20 | 5 | 1 | 0 | 3 | |

***Semi-quantitative test results***

The semi-quantitative tests were conducted on 37 samples purchased via the Internet and at retail shops; however, since all the samples were *stx*-negative, it was not possible to estimate contamination level.

***Calculation results for detection limit***

Detection limits of the method to detect *Shiga* toxin genes based on qualitative and semi-quantitative tests using four O157 strains are presented in Table 3. *Shiga* toxin genes detected by PCR are tick-framed. Among samples with *Shiga* toxin genes detected, the smallest number of inoculum bacteria was 40±2 CFU/5g. Hence the detection limit of these tests was estimated to be 8 CFU/g.

Table 3. Detection of *Shiga* toxin genes by PCR number of O157 inoculum bacteria in 5 g of bovine offal

| Strain (isolation source) | Number of inoculum bacteria | | | | |
|---|---|---|---|---|---|
| | $10^4$ | $10^5$ | $10^6$ | $10^7$ | $10^8$ |
| 204 (human) | +++ | +++ | 384±21 | 40±2 | 4±1 |
| 466 (beef) | +++ | +++ | 219±9 | 22±2 | 2±1 |
| 470 (beef) | +++ | +++ | 252±21 | 27±2 | 3±0 |
| 117 (beef) | +++ | +++ | 246±18 | 26±2 | 3±1 |

**Discussion**

We examined EHEC contamination of bovine offal sold via the Internet. When purchasing the samples, we made adjustments so as to reduce sampling bias associated with selected body part and region (see sub-study by Sawada). Regarding body part, although there is some variability in number, since multiple samples were purchased from the same shop, at least 15 or more samples were made available for each body part, except for heart. Regarding region, since the shops dealing in bovine offal are concentrated in western Japan, it was difficult to purchase samples randomly from all parts of the country; however, nearly the same number of samples was obtained from each prefecture other than Akita and Tokyo.

Shiga toxin genes were detected in 20 samples, which made up 11.2% of 179 samples. By body parts, Shiga toxin genes were detected in 10.0% or more of the samples of heart, liver, rumen (first stomach), reticulum (second stomach), abomasum (fourth stomach) and small intestine, which suggested the possibility of EHEC contamination in a wide range of offal bovine. Among them, detection rate was above 15% for rumen and small intestine, which suggested particularly high EHEC contamination rates in these parts. Regarding heart, since only 3 samples were available, the actual figure is estimated to be lower.

Among samples positive for *Shiga* toxin as well as for O157 or O26; EHEC strains were successfully isolated from only 3 samples, which made up 1.68% of 179 samples. Isolation rate of EHEC strains was 15.0% (3/20) among *Shiga* toxin positive samples, and bovine offal was infected by EHEC but in most cases by either killed bacteria or a very small number of bacteria. Taking into account that detection limit of the testing methods used in this survey was estimated at 8 CFU/g, it is highly probable that even though the samples were infected by EHEC, most bacteria were not isolated either killed at some stage between slaughtering and distribution, or viable bacteria severely damaged for recovery.

O157 was isolated from 2 samples of small intestine, and O26 from omasum. Though contamination level could not be estimated by semi-quantitative tests, considering the calculated detection limit of 8 CFU/g, these samples were presumably contaminated by 8 CFU or more of viable bacteria. Particularly, with high prevalence of *Shiga* toxin genes at 17.1%, and O157 strains also isolated; both prevalence and contamination level of small-intestine were suggested to be higher than that of other parts.

## 2-2. Risk assessment model for enterohemorrhagic E. coli infection from beef

### Overall structure of the model

We simulated exposure routes through consumption of beef at home and restaurants. Cross-contamination exposure was excluded from risk assessment considering that contamination level and contamination frequency of O157 in beef are extremely low, and that cross-contamination has little effect on assessment of relative risk for different consumption patterns assuming it occurs to the same degree in any consumption patterns.

Overall structure of the developed risk assessment model is shown in Fig. 2-2. As explained above, the model covers consumption and subsequent processes as shown in Table 2-1.

Table 2-1. Outline of each stage of risk assessment model

| Stage | Model outline |
|---|---|
| Consumption stage (Exposure) | Consumers are exposed to O157 by consuming "insufficiently cooked or raw beef" which was contaminated and brought home or to restaurants via distribution stage. |
| Disease stage | Consumers exposed to O157 develop illness according to the number of bacteria and dose-response curve. |

We simulated 8 consumption patterns by 'place of consumption', 'consumed beef part' and 'consumption method' as described in Table 2-2.

Table 2-2 Outline of consumption patterns

| Place | Beef part | Consumption method | Description |
|---|---|---|---|
| Home | Dressed carcass | Cooked | Consumption of cooked dressed carcass at home; includes barbecue, hamburgers, steaks, *sukiyaki*, and etc. |
| | | Raw | Consumption of raw dressed carcass at home; includes *yukhoe* (spiced raw beef), and etc. |
| | Offal | Cooked | Consumption of cooked offal at home; includes *horumon-yaki*, *motsunabe* (hot offal soup), and etc. |
| | | Raw | Consumption of raw offal at home; includes liver *sashimi*, omasum *sashimi*, and etc. |
| Restaurant | Dressed carcass | Cooked | Consumption of cooked dressed carcass at restaurants; includes barbecue, hamburgers, steaks, fast food hamburgers, *sukiyaki*, and etc. |
| | | Raw | Consumption of raw dressed carcass at restaurants; includes *yukhoe*, and etc. |
| | Offal | Cooked | Consumption of cooked offal at restaurants; includes *horumon-yaki*, *motsunabe*, and etc. |
| | | Raw | Consumption of raw offal at restaurants; includes liver *sashimi*, omasum *sashimi*, and etc. |

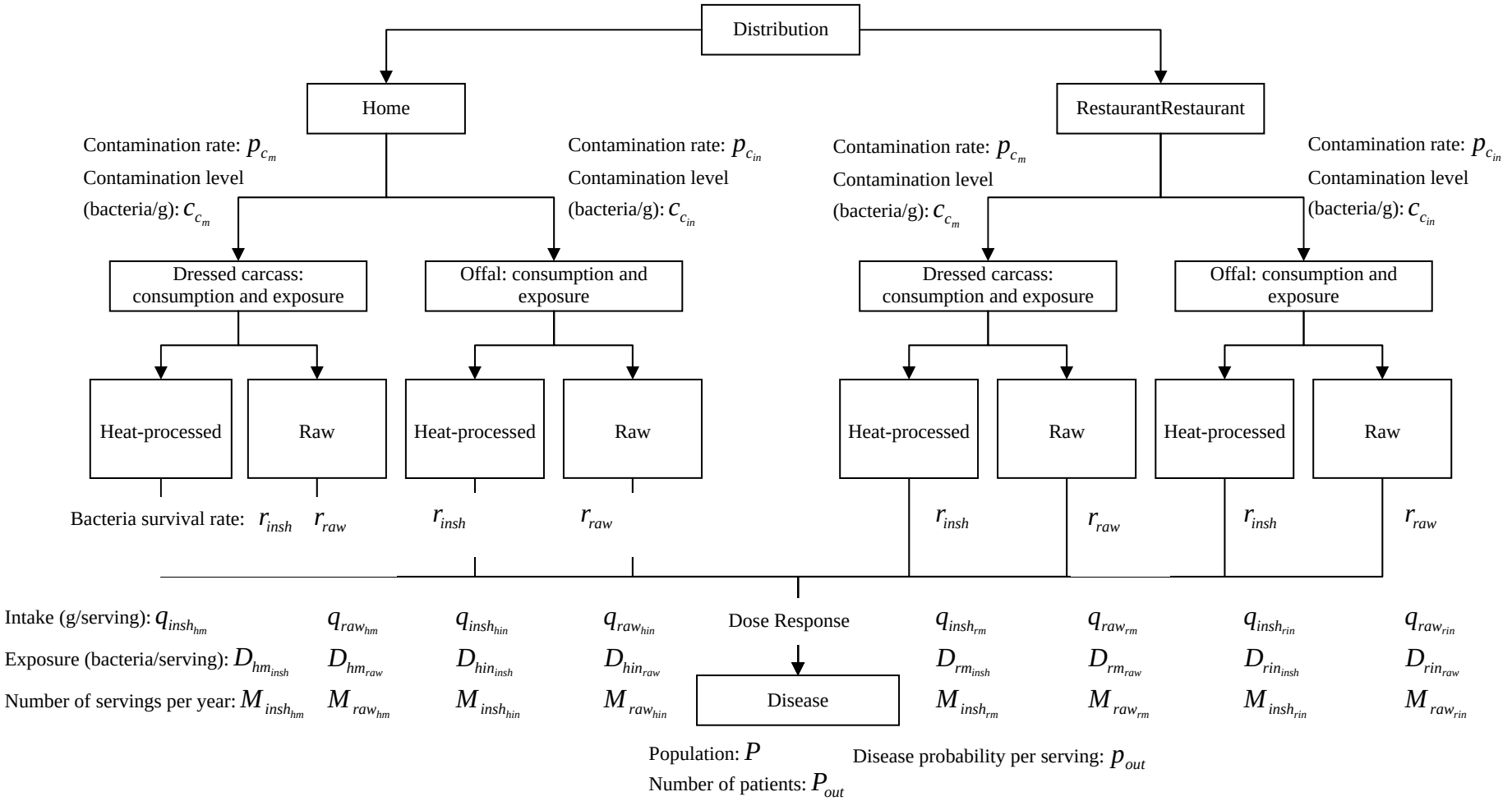



Fig. 2-2. Overall structure of risk assessment model

**Model development and risk estimation**

Here we show the structure of probabilistic model developed in this sub-study as well as its premises and assumptions. This probabilistic model was created using Microsoft Excel and the risk analysis add-on software @RISK5.5 (Japanese version).

***Prevalence***

Regarding contamination of beef brought home or to restaurants via distribution stage, we used the data of *Survey on Actual Conditions of Food Poisoning* (1999-2008) conducted by the Ministry of Health, Labour and Welfare. As for offal, we used the data of our own survey, *2010 Survey on Actual Conditions of Bovine Offal Contamination*. The data on contamination of dressed carcass and offal are given below.

Table 2-3. Contamination of dressed carcass

| | Number of samples | Number of isolated bacteria | Isolation rate |
|---|---|---|---|
| Minced meat (beef) | 1,799 | 0 | 0.00% |
| Cut steak meat | 1,221 | 1 | 0.08% |
| Cubed steak meat | 346 | 0 | 0.00% |
| Bonded beef | 666 | 1 | 0.15% |
| Beef tataki (seared beef) | 829 | 0 | 0.00% |
| Roast beef | 490 | 0 | 0.00% |
| Beef sashimi | 24 | 0 | 0.00% |
| Beef for yukhoe | 11 | 0 | 0.00% |
| Beef | 50 | 0 | 0.00% |
| Total | 5,436 | 2 | 0.04% |

Table 2-4. Contamination of offal

| | Number of samples | Number of isolated bacteria | Isolation rate |
|---|---|---|---|
| 2010 Survey on bovine offal contamination | 229 | 3 | 1.31% |
| Beef liver (for raw consumption) | 179 | 3 | 1.68% |
| Beef liver (for processing) | 19 | 0 | 0.00% |
| Beef liver (for cooking) | 662 | 4 | 0.60% |
| Beef liver (other than for raw consumption) | 67 | 0 | 0.00% |
| Beef liver (other than for cooking) | 229 | 2 | 0.87% |
| Beef liver (unclassified) | 31 | 0 | 0.00% |
| Omasum for cooking | 5 | 1 | 20.00% |
| Total | 1,421 | 13 | 0.91% |

***Contamination level***

Data on O157 contamination level of beef are reviewed at the Ashtown Food Research Center. Using the data by O'Brien (2005), we assumed contamination level of dressed carcass (beef *trimmings*) is identically distributed in the range of $0.7\log_{10}$CFU/g to $1.61\ \log_{10}$CFU/g. Since there is no data on contamination level of offal, we assumed that the contamination level of offal is the same as that of dressed carcass.

***Intake per serving***

We estimated the amount of beef consumed per serving from a questionnaire survey conducted by the Food Safety Commission (2006) for consumption at home, and from a survey on barbecue restaurants for consumption at restaurants. We assumed that barbecue restaurants are representative of all restaurants, although this assumption is not strictly correct. In addition, the mentioned survey by Food Safety Commission (2006) does not include consumption of raw meat; hence we estimated amount of raw meat consumption for both home and restaurants using the mentioned survey on barbecue restaurants. Other parameters of consumption behavior were set based on previous studies and discussion held in our research team. More details can be found in the report of sub-study by Dr. Hasegawa.

***Survival rate of O157***

Bacterial survival rate $r_{insh\_surv}$ due to insufficient cooking was described using the following triangular distribution, based on USDA (2002). Contamination level in case of insufficient cooking is obtained by multiplying this bacterial survival rate by contamination level of beef.

Table 2-5. Probabilistic model for bacterial survival rate in case of insufficient cooking

| Item | Calculation formula |
|---|---|
| Bacterial survival rate in case of insufficient cooking $r_{insh\_surv}$ | $r_{insh\_surv} = RiskTriang(10^{-6}, 10^{-3}, 10^{-1})$ |

***Probability of illness per serving***

When exposure per serving is calculated, a dose-response curve describing relationship between the number of inoculum bacteria $D$ and probability of developing a disease $p_{out}$ is needed to estimate probability of illness per serving. We adopted the following beta-binomial model used by Strachan et al. (2005), for O157 dose-response curve. The following four incidence data cited by Strachan et al. (2005) were used as the model parameters.

1. UK, New Deer, Sheep faeces/soil, Dose=14, Total=228, Infected=20.
2. Japan, Morioka, Salad/seafood, Dose=31, Total=871, Infected=215.
3. USA, Oregon, Deer jerky, Dose=10000, Total=12, Infected=10.
4. Japan, Kashiwa, Melon, Dose=1100, Total=71, Infected=32.

Table 2-6. Dose-response curve

| Item | Calculation formula |
|---|---|
| Dose-response curve | $p_{out} = 1 - \left(\frac{D}{\beta}\right)^{-\alpha}$<br>$\alpha = 0.1619 \quad \beta = 8.1545$ |

Probability of illness per serving at home or a restaurant is estimated by setting the respective exposure $D_h$ or $D_r$ on this dose-response curve. However, exposure per serving is calculated by the total number of bacteria, not separately by beef part (dressed carcass or offal) per serving, or by method (cooked or raw). On the other hand, the objective of this sub-study is to assess probability of illness at home and restaurants, separately by beef part (dressed carcass or offal), and by method (cooked and raw). Thus

we estimated probability of illness per serving for different consumption patterns by prorating in proportion to the level of exposure by each consumption pattern, assuming that the probability of illness is proportional to exposure. This assumption is not strictly correct because the relationship between the probability of illness and exposure is not linear; however, probability of illness increases when the exposure increases. Thus we considered the assumption is acceptable in relative comparison of probability of illness among each consumption pattern.

***Calculation of annual number of consumptions and cases***

Annual number of cases per consumer can be estimated by multiplying the probability of illness per serving by annual number of servings per person. Annual number of cases can be obtained by multiplying annual number of cases per consumer by the population of Japan (127,767,994 as of 2005, Japan Census).

Data on annual number of meals by consumption patterns is required in order to estimate annual number of cases by consumption pattern. However, there are no direct data on the annual number of meals by consumption pattern; thus we used the data of questionnaire survey by the Food Safety Commission (2006), our consumption survey at barbecue restaurants, and 23rd questionnaire on Food Safety and Security in Saitama prefecture (2010).

***Relative risk assessment by consumption patterns***

In order to assess relative risk of the eight consumption patterns, we carried out Monte Carlo simulations with 1 million runs for probability of illness per serving by each consumption pattern. The simulations were performed by the Latin hypercube method using @RISK4.5.5 by Palisade Corp. (Japanese version).

Results of the simulations are presented below. Here, relative probability of illness is defined as the risk of developing the disease per serving for each consumption pattern. When the relative risk of developing a disease per serving for consumption of ‘cooked dressed carcass at home’ is 100, the relative risk of consuming ‘raw offal at a restaurant’ was 350 times higher. In contrast, relative risk of consumption of ‘cooked dressed carcass at a restaurant’ was the lowest.

Annual number of cases was estimated to be roughly 150 thousand. It should be noted that this number includes latent cases not diagnosed at medical institutions, and therefore is not compatible with food poisoning statistics and other data.

| Consumption pattern | | | Risk of developing the disease per serving | |
|---|---|---|---|---|
| | | | Mean | Relative risk |
| Home | Dressed carcass | Cooked | 0.00033% | 100 |
| | | Raw | 0.00663% | 1,993 |
| | Offal | Cooked | 0.00037% | 110 |
| | | Raw | 0.04649% | 13,974 |
| Restaurant | Dressed carcass | Cooked | 0.00010% | 30 |
| | | Raw | 0.00638% | 1,916 |
| | Offal | Cooked | 0.00043% | 128 |
| | | Raw | 0.11837% | 35,577 |
| Annual number of cases (for reference) | | | 150,101 | |

## 3. Application of quantitative risk assessment to setting microbiological metrics of food products

### 3-1. Applicability of quantitative risk assessment to setting microbiological metrics

**FSO using VaR**

When such index as the number of ingested bacteria per serving is described by a distribution as a result of quantitative risk assessment, it is plausible to apply VaR (99 percentile or 99.9 percentile) of the distribution as the “maximum value” and set it as FSO (or PO at stages close to intake of food) in the field of food safety, since this approach has been taken by financial and other institutions for quantitative risk assessment.

On the other hand, CVaR used by financial and other institutions as a reference to risk assessment, has information value in the sense that it focuses on the further tail of distribution than VaR; however, adoption of CVaR is not necessary at this moment, since FSO and PO, as maximum values not to be exceeded, do not require information on the risk beyond the values.

**Development from FSO and PO to quantitative risk assessment model in the food chain**

In the field of food safety, performance objectives must be set at every stage of the food chain. However, among quantitative risk assessments in other fields, we did not find any examples of using FSO, or PO at stages close to intake of food, to develop PO at final stages. Thus we considered a method to develop scenario analysis investigated in the previous year into metrics for intermediate processes in the food chain. Fig. 3 illustrates the concept of development from FSO to PO of final stages of the process using the scenario analysis. The procedure is described below (the numbers correspond to those in the diagram):

(1) VaR is confirmed as a result of quantitative risk assessment at the stage of intake of food.

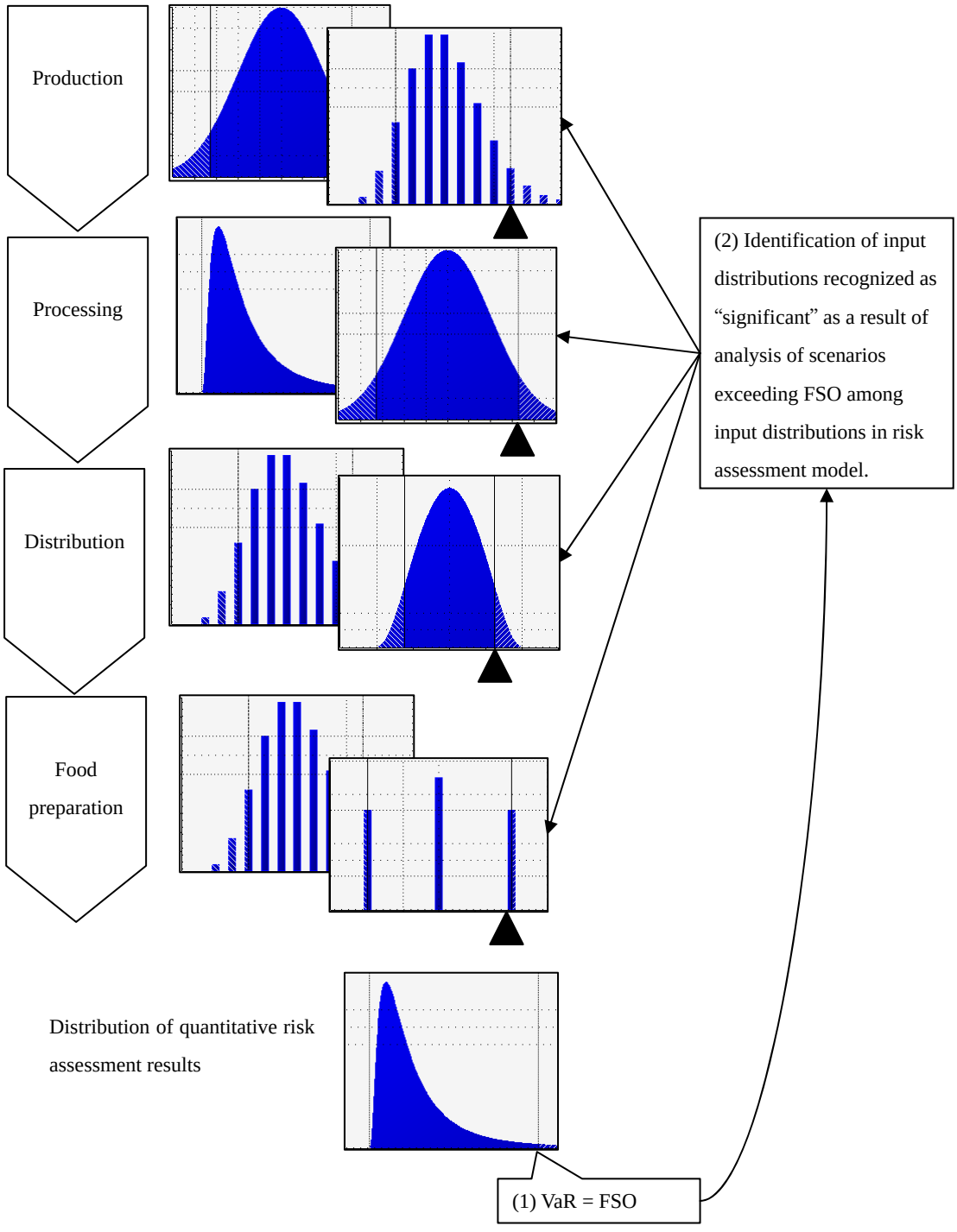
(2) Scenario analysis is applied to scenarios in which input distributions at each stage of the food chain exceed the VaR confirmed in (1), and a “significant” input distribution is identified. (For explanations on scenario analysis, see research content and results of FY 2009).

(3) Median value is calculated for the sub-set of scenarios exceeding FSO among the input distribution identified in (2).

(4) Stress analysis or other methods are used to verify whether the value in (3) is acceptable as PO.

Stress analysis in Monte Carlo simulations is a method to estimate the effect of stress from a

statistical point of view, by comparing simulation result in which stress (within a certain value range in simulations) is applied to a specific input distribution, with simulation result without stress. In the above procedure (1) to (4), stress is determined in the range beyond the input distribution PO calculated in (3); and the stress analysis enables verification of assumed FSO by comparison with statistical value with stress.




(3): Median value of subset (shown by ▲ in the diagram)

(4) Stress analysis etc. is conducted to verify whether the value ▲ is acceptable as PO

Concept of development from FSO to PO of upstream processes using scenario analysis

**3-2. Development of training materials for 'Probabilistic microbiological risk assessment'**

Since Monte Carlo method is essential for quantitative risk assessment, we used @RISK5.5 Professional (Japanese version) by Palisade Corporation (U.S.) in this training. The contents and time schedule of the two-day preliminary course developed and implemented are given in the table below.

Content of preliminary course

| | Item | Content | Number of slides | Number of practical | Time required (hours) |
|---|---|---|---|---|---|
| 1st day | 1 | Framework of risk analysis | 9 | - | 0.5 |
| | 2 | Procedure of risk assessment | | - | |
| | 3 | Risk modeling in the food chain | | - | |
| | 4 | Basics of probability and statistics theory | 7 | - | 0.5 |
| | 5 | Monte Carlo simulations | 96 | 3 | 4.5 |
| | 6 | Procedure of risk analysis using the Monte Carlo simulation tool @RISK | | | |
| 2nd day | 7 | Estimation of distributions based on data and expert opinions | 24 | 4 | 1.5 |
| | 8 | Stochastic processes and theorems used in risk analysis | 19 | 3 | 2.5 |
| | 9 | Bayesian estimation | 10 | 1 | 1.0 |
| | 10 | Dose-response models | 17 | - | 0.5 |

**3-3. Estimation of prevalence and disease burden of enterohemorrhagic E. coli, dysentery and cholera in Japan**

Conservative (underestimated) and liberal (overestimated) number of cases obtained using point estimate are given in the table below.

Point estimation for enterohemorrhagic *E. coli*, dysentery and cholera

| | VTEC | | Cholera | | Dysentery | |
|---|---|---|---|---|---|---|
| | Min | Max | Min | Max | Min | Max |
| bloody | 80860 | 622300 | | | | |
| non-bloody | 139975 | 2666196 | | | | |
| Total | 220,835 | 3,288,496 | 1,031 | 7,856 | 10,181 | 76,794 |

**3-4. Contingent valuation method (CVM) study on factors affecting willingness-to-pay (WTP)**

In this study, we found out that WTP for health maintenance of anterior teeth region and molar region was, respectively, 35,849 yen and 43,100 yen on average; in both cases, WTP depended on age, educational background, household income, past experience of dental treatment, etc.

**Discussion and conclusions**

It is ideal if the input data in the models are precise as possible in order to implement quantitative risk assessment with high accuracy. In practice, however, sufficient data for microbiological risk assessment are rarely obtainable. For example, in case of *Campylobacter*, introduction of data on contamination level before and after slaughtering resulted in substantial change in order of the effectiveness of different measures against poisoning. In case of O157, too, availability of series of necessary data is limited at this stage, and risk assessment models are developed based on many assumptions. In order to estimate effects of data accuracy on

quantitative risk assessment, we conducted simulations, to see the effect of beef contamination rates, of which the accuracy of the data is considered especially low. We found out that even a slight increase in the number of samples with bacteria isolated exerted a dramatic effect on risk assessment results. The amount and frequency of beef consumption is also classified into several consumption patterns based on many assumptions, and more data are expected urgently.

Such limitations in data and models must be taken into account when interpreting the risk assessment results. Reviewers with knowledge on probabilistic risk assessment and underlying techniques are needed for this reason. Training materials developed by this research team are expected to contribute to training reviewers.

**Overall results and conclusion obtained through FY 2008 - 2010**

**Results**

**1. Risk assessment model for Vibrio parahaemolyticus infection from horse mackerel**

The best-case scenario in this model (washing fish at the port or market, sterilization of water during transportation, transportation and storage at low temperature, washing fish visceral cavities during preparation), gave a mean number of nine ingested *Vibrio parahaemolyticus* , and the mean probability of illness of $6\times10^{-6}$ per meal containing raw horse mackerel. Not washing visceral cavities during preparation had the greatest effect by increasing the mean probability of illness (or the number of ingested pathogenic bacteria) by 15 times. The mean probability of illness increased by 50% if transportation involves high-temperature period, and by 7% if fish is not washed. The water used for transportation and storage had a negligible effect. With the worst-case scenario, the mean number of bacteria per serving of horse mackerel was 230, and the mean probability of illness was $1.4\times10^{-4}$.

Although the values are based on assumptions and experimental data in previous studies, the results are compatible with actual reported number of food poisoning cases as follows. Based on the amount of raw horse mackerel consumption in Japan, total annual number of pathogenic *Vibrio parahaemolyticus* ingested is estimated at $1.7\times10^{9}$ in the best-case scenario. Because of the linearity of the dose-response relation, annual number of cases can be estimated at about $10^{3}$ in the best-case scenario, irrespective of the distribution of ingested bacteria. This is of the same order of magnitude as the reported annual number of food poisoning patients by *Vibrio parahaemolyticus* (1,000 to 3,000 persons). Considering the proportion of reported cases in all food poisoning cases is estimated to be 1 out of 20 (Kubota et al. 2007), this estimate appears to be compatible with actual number of cases.

The dose-response relationship used in this estimation was determined by the USFDA from old human feeding trial data for risk assessment in raw oysters. In case of oysters, the adjustment factor of 1/27 was used to adjust to epidemiological data. It was not used in horse mackerel because adjustment factor is specific to the food.

The following data are required to estimate the annual number of food poisoning cases caused by combination of this pathogen and food using the quantitative model for process from fishing to consumption.:

(1) Densities of *Vibrio parahaemolyticus* on the surface and in the visceral cavity of horse mackerel at landing;
(2) Proportion of pathogenic strains to the total number of *Vibrio parahaemolyticus*;
(3) Occurrence frequency of different scenarios in each process;
(4) Quantity and frequency of consumption;
(5) Dose-response relationship for different population groups for each pathogenic strain.

However, only a part of such information is available. The data underlying the results may not necessarily represent reality. Particularly, the dose-response relationship including the risk assessment by USFDA involves great uncertainty), which brings about uncertainty of many digits in the incidence estimation.

However, our model, starting with bacterial densities at landing and involving the processes of contamination, growth in fish and during preparation, confirmed that the bacterial count of *Vibrio parahaemolyticus* ingested through raw horse mackerel lies within the range where the dose-response relationship remains linear, as

estimated from available data on bacterial densities and proportion of pathogenic strains (in this case, it is sufficient to consider pathogen intake per total population). Even in the dose-response relationship with the highest infection rate, which takes account of statistical uncertainty of the underlying data, the bacterial count resulting in the infection risk of 10% is $10^5$, and one can assume linearity up to this level. The model estimated the number of ingested bacteria lies within this range. Therefore, the conclusion derived from comparison of measures against food poisoning using the dose-response model (washing of fish at landing, sterilization of water in transportation, transportation and storage at low temperature, and washing of fish body cavities during food preparation) is that they do not rely on dose-response relations except that linearity holds. In addition, the assumed initial bacterial densities and proportion of pathogenic bacteria may affect the number of cases, but not comparison of the control measures.

Thus, it was demonstrated that food preparation and temperature control are important for prevention of contamination of *sashimi*, and that risk assessment based on available information is useful enough to prevent food poisoning.

**2. Quantitative risk assessment model for Campylobacter food poisoning from raw poultry**

We improved the quantitative risk assessment model developed by FY 2009 for food poisoning from raw poultry meat infected by *Campylobacter*, by modeling the change in contamination level at a slaughterhouse, dividing incompleteness of farm surveys from adoption rate of strategic poultry processing, and modeling cross-contamination rate at a slaughterhouse.

Regarding the base case of probability of infection per serving of poultry dish for those who eat raw meat and those who do not, the modified model produced much the same results as the model developed by FY 2009. On the other hand, while the previous model suggested dramatic reduction of infection risk by reduced rate of raw food consumption for those who eat raw food, the modified model showed the risk reduction effect of reduced on-farm contamination rate was higher. In addition, strategic poultry processing also showed the strong effect, at 3 to 4% per 10% of adoption rate. The effect of strategic processing was reduced with lower test sensitivity due to cross-contamination caused by infected poultry with a false negative test result. For those who do not eat raw meat, the previous model suggested that reduced cross-contamination risk during food preparation resulted in bigger reduction of infection risk as compared to other measures against food poisoning, while the risk reduction effect of reduced on-farm contamination rate proved higher in the modified model. Just as with those who eat raw food, the effect of strategic processing was reduced with lower test sensitivity.

**3. Development of risk assessment model for enterohemorrhagic E. coli infection from beef**

Beef consumption patterns were divided into 8 groups by ‘consumption place’ (home/restaurant), ‘consumed beef part’ (dressed carcass/offal), and ‘consumption method’ (cooked/raw); and Monte Carlo simulations of probability of illness per serving were conducted for each consumption pattern for relative risk assessment.

Here, relative probability of illness is defined as the risk of developing the disease per serving for each consumption pattern, when the probability of disease per serving by consuming ‘cooked dressed carcass at home’ is 100.

The risk was the highest when consuming ‘raw offal at restaurant’, 350 times higher as compared to consumption of ‘cooked dressed carcass at home’. In contrast, the risk was the lowest when consuming ‘cooked dressed carcass at restaurant’.

The annual number of cases was estimated to be 150,000. It should be noted that this number includes latent cases not diagnosed at medical institutions, and therefore is not compatible with food poisoning statistics and other data.

**Discussion and conclusions**

In the current situation, available quantitative or semi-quantitative data for risk assessment are very limited. For this reason, uncertainties in risk assessment may cast a huge impact upon the results, as is the case with semi-quantitative risk assessment. However, it is not known what kind of data are lacking until a model is created and risk assessment is conducted. The future research is expected to be conducted thorough both data generation, that is, survey of actual conditions and experimental study, and practical risk assessment.

In order to use statistical analysis tools and other software for multivariate analysis, regression analysis, variance analysis, time series analysis and Markov chain Monte Carlo method, extension modules or individual programming are required. When considering the use of such tools, it is necessary to define, in advance, specific functions needed for quantitative risk assessment of food products, and then to evaluate the tool from several standpoints such as applicability of required analysis methods, the level of maintained accuracy, compatibility, and etc. When dose-response relationship is non-linear or non-monotonic, and history dependence is important, qualitative discussion or non-probabilistic quantitative approaches become difficult or even impossible. Under such circumstance, probabilistic methods gain importance, and necessity grows for exact and a wider range of information on strains, bacterial counts, does-response relationships, exposure histories, and etc.

In this sub-study, we created a model for relative risk assessment by beef consumption pattern, and showed quantitatively that the risk of eating raw offal is extremely high as compared to other consumption patterns. We also found that accuracy of various data incorporated into the model must be improved in order to improve the accuracy of the model and quantitatively assess absolute risk such as the annual number of patients . Particularly, contamination rates of beef were estimated by a survey with a very limited number of samples, which presumably does not represent fully the actual conditions of the whole population (all beef at retailers). In addition, the amount and frequency of beef consumption had to be put into patterns based on many assumptions, and more data on beef consumption are needed urgently.

**Papers and magazines published based on this research**

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**Future issue**

There are no unified directions and methods in risk assessment of microorganisms, such as ADI in risk assessment of chemicals. Presently, risk magnitude and effectiveness of possible control measures are estimated based on available data and information, upon request of risk management institutions.

As shown in this study, data on microbial contamination and consumer behavior are insufficient in many cases; hence probabilistic techniques are used to compensate for data variability and uncertainty. For this reason, risk assessment cannot be carried out by microbiologists only, and a team for risk assessment must be made up of specialists in various fields of science and engineering.

However, few people comprehend specific aspects of risk assessment using probabilistic methods, and a very few researchers are capable of actual probabilistic risk assessment. By contrast, when probabilistic risk assessment draft is proposed, risk assessment by the U.S. Government involves technical peer reviews by experts in related fields as well as by research institutions and risk management divisions of related governmental agencies.

As directions of future research in assessment of the effects of food on health, continued collection of data necessary for risk assessment is essensial. However, data collection alone is not sufficient for risk assessment, and we expect that further studies are promoted in the area of quantitative approach and functionalization of collected data as well as risk estimation; and that this research will contribute to deeper understanding of risk assessment techniques.

**List of Collaborators**

The following collaborators voluntarily participated in meetings and discussions of the research team, collection and organization of data, development of risk models, and reporting, for 1 to 3 years. A total of 14 persons including 7 listed below contributed to this research.

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