# *Listeria monocytogenes* in foods

## Executive Summary

Food Safety Commission of Japan

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment of *Listeria monocytogenes* (LM) in ready-to-eat (RTE) foods contaminated with LM. Data used for the assessment were those of food intake, the susceptible people population, estimated incidence of human listeriosis, and LM contamination in RTE foods in Japan. Contamination levels of LM in RTE foods and exponential dose-response model described in the evaluation report of Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) were also used for the assessment. The result of the assessment shows that if the contamination level of LM in RTE foods is below 10,000 CFU/g at consumption, the number of invasive listeriosis cases would be lower than those (200 cases) calculated from data of surveillance conducted by the Ministry of Health, Labour and Welfare. Therefore, the development of invasive listeriosis in Japan is likely caused by consumption of foods contaminated with LM at a level higher than 10,000 CFU/g. Furthermore, the result indicates that if some foods are contaminated with such an extremely high level of LM as 1,000,000 CFU/g at consumption, the number of patients would increase with the increase in the proportion of such heavily contaminated foods even when contamination levels in foods are relatively low as a whole. Therefore, to reduce the incidence of listeriosis, it is necessary to decrease the frequency of occurrence of foods contaminated heavily with LM by specifying a storage period for RTE foods. Manufacturers and processors of RTE foods should implement end product testing on LM, good hygienic practices, and environmental monitoring for LM in their plants.

## Conclusion in Brief

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment toward the establishment of standards of *Listeria monocytogenes* (hereafter referred to as LM) in foods based on scientific data accumulated so far, estimated incidence of human listeriosis, and the status of LM contamination in foods distributed in Japan. The assessment covers the following targets (1) pathogen: LM, (2) population: all people living in Japan (healthy and susceptible populations), (3) disease: invasive listeriosis caused by oral exposure to LM, and (4) foods: foods prepared to be consumed without further heating (ready-to-eat foods: RTE foods). Human listeriosis can be grouped in non-invasive listeriosis characterized by gastroenteritis, and invasive listeriosis by meningitis. The assessment covers invasive listeriosis which accompanies relatively severe symptoms and can be more reliably diagnosed than non-invasive listeriosis.

Since a large number of outbreaks of listeriosis associated with foods had been reported in other countries, FSCJ decided to conduct the risk assessment focusing on RTE foods, taking into account the status of LM contamination of foods in Japan and other countries as well as the influence on the risk of food preparation and consumption characteristics. The annual number of patients with listeriosis in Japan was estimated using the method of Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) including exponential dose-response model ($P = 1 - e^{-rN}$). In addition to data collected in Japan on food intake, a susceptible population, and food contamination with LM, data of the contamination levels of LM in RTE foods described in JEMRA's evaluation report were used for the estimation, con-

sidering that data obtained in Japan had been included in the JEMRA's data. Furthermore, the multiple dose approach based on various levels of LM contamination in RTE foods at consumption was used for the estimation, because multiple doses were regarded to reflect the actual situations. In the risk assessment, this estimate of the number of patients was compared with 200 cases which was the number calculated as the annual number of cases of listeriosis from data of Japan Nosocomial Infections Surveillance (JANIS) established by the Ministry of Health, Labour and Welfare and therefore considered to represent the current status in Japan.

The result of the assessment shows that if the contamination level of LM in RTE foods is below 10,000 CFU/g at consumption, the number of listeriosis cases would be lower than that calculated from the JANIS data (200 cases). Furthermore, the risk of developing listeriosis is considered to be extremely low in a healthy population. Therefore, the development of listeriosis in Japan is likely caused by consumption of foods contaminated with LM at a level higher than 10,000 CFU/g. Based on the quantitative data on LM in retail foods in Japan, although the quantitative data is limited, it is considered that foods consumed by patients could contain extremely high levels of LM. Such foods are, for example, the foods that can support the growth of LM and are stored in a refrigerator for a relatively long period of time.

Referring to JEMRA's report, FSCJ estimated the number of patients with listeriosis when foods contaminated with such an extremely high level of LM as 1,000,000 CFU/g are consumed. The result indicates that if some foods are contaminated with extremely high levels of LM at consumption, the number of patients would increase with the increase of the proportion of such heavily contaminated foods even when contamination levels in foods are relatively low as a whole. To reduce the incidence of listeriosis, therefore, it is necessary to decrease the frequency of occurrence of foods contaminated with LM at the extremely high levels of LM through control measures including specification of a storage period, taking into account that LM can grow in foods during cold storage even at or below 4°C. Furthermore, this assessment indicates that contamination and growth of LM in RTE foods could occur during the manufacturing and processing of them, because LM is widely dispersed in the environment, can grow at refrigeration temperature, and survives for a long period of time in the food manufacturing environment. Therefore, FSCJ considers that manufacturers and processors of RTE foods should not only conduct the end product testing on LM, but also implement good hygienic practices to control LM in the plant environment and environmental monitoring including environmental testing for LM for verification. The implementation of these control measures will enable not only the prevention of contamination of RTE foods and manufacturing equipments with LM derived from the environment but also the suppression of growth of the contaminated LM, and thereby decrease the frequency of LM contamination of RTE foods.

JEMRA estimated that the risk of developing listeriosis in the susceptible population with impaired immune functions is approximately 200 times higher than that in the healthy population. Consistent with it, the result of the analysis of the JANIS data shows that most listeriosis cases in Japan have occurred among elderly populations aged 65 or older accounting for 77.6% of all the cases. FSCJ therefore considers that development and implementation of risk management measures focusing on the susceptible population as well as verification of its effectiveness will be effective in reducing the risk of LM infection.

## Risk Assessment

FSCJ conducted a risk assessment toward the establishment of standards for *Listeria monocytogenes* (LM) based on scientific data accumulated so far in response to the request from the Ministry of Health, Labour and Welfare.

Human listeriosis can be grouped in non-invasive listeriosis characterized by gastroenteritis, etc., and invasive listeriosis by meningitis, etc. The present assessment covers invasive listeriosis, which accompanies relatively severe symptoms and can be more reliably diagnosed compared with non-invasive listeriosis. Since a large number of outbreaks of listeriosis associated with foods had been reported in many other countries, FSCJ decided to conduct the risk assessment focusing on RTE foods, taking into account the status of LM contamination of foods in Japan and other countries as well as the influence on the estimation of risks of food preparation and consumption characteristics.

FSCJ estimated the annual number of patients with human listeriosis using the method of JEMRA (exponential dose-response model ($P = 1 - e^{-rN}$), and constants) with the r-value of $1.06 \times 10^{-12}$ and $2.37 \times 10^{-14}$ for susceptible and healthy populations, respectively. Then the estimated number of listeriosis was compared with the annual number of cases of human listeriosis that had been calculated from data of JANIS to be 200, which was regarded to represent the current status of listeriosis in Japan. To estimate the annual number of listeriosis cases using the JEMRA method, the ingested dose in terms of the number of LM was estimated by multiplying the LM levels in RTE foods at consumption by the size of serving. Then, the risk of development of listeriosis was calculated from the ingested dose using the ex-

ponential dose-response model, and the calculated risk was further corrected with the proportion of contaminated RTE foods, the percentage of the susceptible population, etc. Finally, the annual number of patients with listeriosis in Japan was calculated by multiplying the risk by the total number of servings per year. To estimate the LM levels in RTE foods at consumption, two approaches were used: One is a single dose approach and the other a multiple dose approach. The former assumes contamination of the RTE foods with LM at the same level at consumption, and the latter assumes that the RTE foods are contaminated with LM at various levels at consumption. Based on data collected in Japan, the size of a serving of RTE foods was assumed at 50, 100, and 200g per serving, and the percentage of the susceptible population was assumed to be 27%. Based on the data of a survey of LM contamination of foods in Japan, 2.58% was used as the value of the proportion of RTE foods contaminated with LM. For the contamination levels in RTE foods in terms of the number of LM, JEMRA's data was used due to the lack of sufficient data in Japan, taking into account that JEMRA's data included data from Japan.

The multiple dose approach, based on various levels of LM contamination in RTE foods at consumption, was used for the estimation of the annual number of patients with listeriosis, because multiple doses may reflect the actual situation more likely than a single dose. As a result, the estimated number of patients with listeriosis per year under the assumption that 50g of RTE foods per serving is consumed is:

· Less than 1 in both the susceptible and healthy population groups when LM levels of RTE foods at consumption are 100 CFU/g or below.

· Four (4 in the susceptible group and less than 1 in the healthy group) when LM levels of RTE foods at consumption are 1,000 CFU/g or below.

· Twenty (19 in the susceptible group and 1 in the healthy group) when LM levels of RTE foods at consumption are 10,000 CFU/g or below.

· Ninety-nine (94 in the susceptible group and 5 in the healthy group) when LM levels of RTE foods at consumption are 100,000 CFU/g or below.

The estimated number of patients with listeriosis per year under the assumption that 100g of RTE foods per serving is consumed is:

· One (1 in the susceptible group and less than 1 in the healthy group) when LM levels of RTE foods at consumption are 100 CFU/g or below.

· Eight (8 in the susceptible group and less than 1 in the healthy group) when LM levels of RTE foods at consumption are 1,000 CFU/g or below.

· Forty-one (39 in the susceptible group and 2 in the healthy group) when LM levels of RTE foods at consumption are 10,000 CFU/g or below.

· One hundred ninety-nine (188 in the susceptible group and 11 in the healthy group) when LM levels of RTE foods at consumption are 100,000 CFU/g or below.

The estimated number of patients with listeriosis per year under the assumption that 200g of RTE foods per serving is consumed is:

· Three (3 in the susceptible group and less than 1 in the healthy group) when LM levels of RTE foods at consumption are 100 CFU/g or below.

· Eighteen (17 in the susceptible group and 1 in the healthy group) when LM levels of RTE foods at consumption are 1,000 CFU/g or below.

· Eighty-three (79 in the susceptible group and 4 in the healthy group) when LM levels of RTE foods at consumption are 10,000 CFU/g or below.

· Three hundred ninety-eight (376 in the susceptible group and 22 in the healthy group) when LM levels of RTE foods at consumption are 100,000 CFU/g or below.

From this result, it was found that if the contamination level of LM in RTE foods is below 10,000 CFU/g at consumption, the number of patients with listeriosis will be lower than that calculated from the JANIS data. Furthermore, for the healthy population, the risk of developing listeriosis was considered to be extremely low.

However, since the number of cases of listeriosis in Japan calculated from the JANIS data is 200, the development of listeriosis is likely caused by consumption of foods contaminated with LM at a level higher than 10,000 CFU/g. Based on the quantitative data on LM in retail foods in Japan, although the data is limited, it was considered that foods consumed by patients could contain extremely high levels of LM. Such foods are, for example, the foods that can support the growth of LM and are stored in a refrigerator for a relatively long period of time.

Therefore, referring to JEMRA's report, FSCJ estimated the number of patients with listeriosis when foods contaminated with such an extremely high level of LM as 1,000,000 CFU/g are consumed. Under the situation where contamination levels of LM at the time of consumption are below 10,000 CFU/g in most RTE foods, if the proportion of foods

contaminated with an extremely high level of LM is below 0.0001%, such heavily contaminated foods do not notably change the predicted number of patients of listeriosis. However, the predicted number increases remarkably with the increase in the proportion of the heavily contaminated foods beyond 0.0001%: for example, when the size of a serving is assumed to be 200g, the predicted numbers at the proportion of the heavily contaminated foods of 0%, 0.0001%, 0.001%, 0.01% and 0.1% are 83, 91, 167, 933, and 8,593, respectively, and therefore the number increases 2-, 10- and 100-fold with the increase in the proportion of the heavily contaminated foods from 0% to 0.001%, 0.01% and 0.1%, respectively. This estimation indicates that if some foods are contaminated with extremely high levels of LM at consumption, the number of patients would increase with the increase of the proportion of such foods even when contamination levels in foods are relatively low (<10,000 CFU/g) as a whole. To reduce the number of listeriosis cases, therefore, it is necessary to decrease the frequency of the occurrence of foods contaminated with LM at the extremely high levels of LM through control measures including specification of a storage period, taking into account that LM can grow in foods during cold storage even at or below 4 °C.

Furthermore, this assessment indicates that contamination and growth of LM in RTE foods could occur during manufacturing and processing of foods, because LM is widely dispersed in the environment, can grow at refrigeration temperature, and survives for a long period of time in the food manufacturing environment. FSCJ considers that manufacturers and processors of RTE foods should not only conduct the end product testing on LM, but also implement both good hygienic practices to control LM in the plant environment and environmental monitoring including environmental testing for LM for verification. The implementation of these control measures will enable the prevention of contamination of RTE foods and manufacturing equipments with LM from the environment and the growth of the contaminated LM, and thereby decrease the frequency of LM contamination of RTE foods.

JEMRA estimated that the risk of developing listeriosis in the susceptible population with lowered immune functions is approximately 200 times higher than that in the healthy population. The result of the analysis of the JANIS data shows that most listeriosis cases have occurred among elderly populations aged 65 or older accounting for 77.6% of all the cases. FSCJ therefore considers that development and implementation of risk management measures focusing on the susceptible population as well as verification of its effectiveness will be effective in reducing the risk of LM infection.