

This is a provisional English translation of an excerpt from the original full report.

## Risk Assessment Report

### *Kudoa septempunctata* in olive flounder<sup>1</sup> (Microorganisms and viruses)

Food Safety Commission of Japan (FSCJ)  
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#### ABSTRACT

FSCJ conducted a self-tasking risk assessment of the genus *Kudoa* myxosporea. This assessment focused on *Kudoa septempunctata* (*K. septempunctata*), a specie of the genus *Kudoa* myxosporea. The fish parasite was reported to cause food poisoning and adverse health effects in human. For other species of the genus *Kudoa* myxosporea, there is no sufficient evidence of the adverse health effects in human. FSCJ decided therefore to focus on *K. septempunctata* in olive flounder (*Paralichthys olivaceus*) as a target pathogen and food in this assessment.

Food poisoning outbreaks due to ingestion of *K. septempunctata* have occurred nationwide. The Ministry of Health, Labour and Welfare decided in June 2011 to deal with symptomatic cases caused by ingestion of *K. septempunctata* parasitizing olive flounder as food poisoning cases. Since then, 33, 41, 21 and 43 outbreaks of the food poisoning were reported from June to December 2011, and in 2012, 2013 and 2014, respectively. Diarrhea and vomiting, that are self-healing, were reported as major symptoms.

Disability-adjusted life years (DALYs)<sup>2</sup> of *K. septempunctata* provisionally calculated was extremely low compared to those of *Campylobacter spp.* and Norovirus. This indicates that the disease burden of food-borne *K. septempunctata* poisoning including symptoms, disease duration, severity, prognosis, and sequelae occurrence (though self-healing) is remarkably low compared to that of foodborne *Campylobacter spp.* and Norovirus infections.

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<sup>1</sup> A foodborne disease associated with the consumption of fresh raw fish (olive flounder) became a topic of concern from 15 years ago. The disease manifested as diarrhea and vomiting with a good prognosis, and no causative agent had been identified. MHLW launched a research project on this disease in 2010, and released a notice in 2011 that a new parasite was the apparent causative agent. This risk assessment report is on this parasite in olive flounders. The parasite was shown to be easily inactivated by heating at 80C for over 10 sec or freezing at -20C for 4 hr.

<sup>2</sup> DALY is a measure of overall disease burden expressed as the number of years lost due to ill-health, disability or early death. The DALY is used internationally as a comprehensive and quantitative index of disease burden.

In the food poisoning or symptomatic cases for which the amount of consumed fish and the spore count in fish were reported, total count of spores ingested by a patient was estimated to be approximately over  $10^7$  per person. Ingestion of spores over  $10^7$  is thus considered to elicit symptoms of poisoning such as diarrhea and vomiting, although there would be a possible variation in individual susceptibility.

Japanese local governments investigated origins of olive flounder implicated in 64 outbreaks which occurred in 2013 and 2014. Among the outbreaks, 44 were caused by imported cultured olive flounder, 10 were by domestic natural olive flounder, and one was by domestic cultured olive flounder. Information was not disclosed for two outbreaks, and origins of olive flounder were unclear for 7 outbreaks. The Ministry of Agriculture, Forestry and Fisheries notified the prevention measure against food poisoning by *K. septeempunctata* to domestic fish farms in 2012. A marked decrease in occurrence of food poisoning by *K. septeempunctata* since 2013 indicated that this measure was effective in prevention of the food poisoning. Prevention of the parasite infection in olive flounder in fish farming is therefore considered to be important in reduction of the risk to human health, although life cycle of and infection route to olive flounder have not fully been understood.

According to the provisional calculation of DALYs, burden of disease caused by *K. septeempunctata* is considered to be remarkably low.

Taking into account the provisional DALYs, the risk management organizations are urged to consider possible prevention measures against this parasite. Implementation of the prevention measure in fish farm is continuously crucial. In addition, under the current situation where outbreaks of *K. septeempunctata* poisoning still occur, it is important to continue watching of the trend of the food poisoning. Detailed investigations of the outbreaks are also important to identify the source of contamination, and to determine the amount of consumed olive flounder and spore concentrations in them. Moreover, the prevention measure in fish farms is considered to be effective for imported cultured olive flounder as well as for domestic cultured olive flounder rather than preventive measures depending on test of the parasite contamination in imported fish at the import.

## Executive Summary

The genus *Kudoa myxosporea* is a fish parasite. More than 97 species have been identified to belong this genus as of August 2015. Among them, 20 species have been found in Japan. This assessment focused on *Kudoa septempunctata* (*K. septempunctata*), one of the genus *Kudoa myxosporea*, that was reported to cause food poisoning and adverse health effects in human. For other species of the genus *Kudoa myxosporea*, there is no sufficient evidence of the adverse health effects in human. FSCJ decided therefore to focus on *K. septempunctata* in olive flounder as a target pathogen and food in this assessment.

Food poisoning outbreaks due to ingestion of *K. septempunctata* have occurred nationwide. The Ministry of Health, Labour and Welfare decided in June 2011 to deal with symptomatic cases caused by ingestion of olive flounder parasitized with *K. septempunctata* as food poisoning cases. Since then, 33, 41, 21 and 43 outbreaks of the food poisoning were reported from June to December 2011, and in 2012, 2013 and 2014, respectively. In addition to these outbreaks, considerable numbers of outbreaks have possibly been unreported because of its mild and transient symptoms.

Diarrhea and vomiting, that are self-healing, were reported as major symptoms. Reported incubation periods, the period from intake to onset, range from approximately one to 22 hours.

Pathogenicity of *K. septempunctata* has been studied using cultured cells, suckling mice, and house musk shrews (*Suncus murinus*). Watery diarrhea and intestinal fluid accumulation were observed as signs of diarrheagenicity in suckling mice after intragastric administration of over  $10^6$  spores of *K. septempunctata* per mouse.

Vomiting was observed in the shrews after feeding them with tissues of olive flounder parasitized by *K. septempunctata* at  $4-6 \times 10^7$  spores per g, or after intragastric administration of  $6 \times 10^7$  purified spores per animal. The shrews started to vomit 20 to 30 minutes after the feeding or administration, and then vomited twice or three times during the following one hour.

When spores of *K. septempunctata* were inoculated on Caco-2 cells, a cell line derived from human colon carcinoma, sporoplasms released from the spores intruded into the Caco-2 cells accompanying a decrease in the transepithelial electrical resistance of the cell monolayer. The result indicated that sporoplasms injured the intestinal cells.

The vomit- and diarrhea-eliciting effects observed in experimental animals and the cell-injury observed in cultured cells suggest that spores or sporoplasms of *K. septempunctata* act directly to intestinal cells causing diarrhea or vomit also in human.

The prognosis of the *K. septempunctata* poisoning has been regarded to be good. Provisionally calculated disability-adjusted life years (DALYs)<sup>3</sup> of *K. septempunctata* was extremely low compared to those of *Campylobacter spp.* and norovirus. This indicates that the disease burden of food-borne *K. septempunctata* poisoning including symptoms, disease duration, severity, prognosis, and sequelae occurrence (though self-healing) is remarkably low compared to that of foodborne *Campylobacter spp.* and Norovirus infections.

Detail epidemiological data including the amount of consumed foods, number of *K. septempunctata* spores in olive flounder, and incidence rate of symptomatic case are available only for limited numbers of the outbreaks or cases. The number of the spores per 1 g of implicated olive flounder tissues was reported to be mostly above  $1.0 \times 10^6$ , but the number less than  $1.0 \times 10^6$  was also found in some outbreaks or cases. The amount of ingested olive flounder was also reported for some of the outbreaks or cases. The ingested amount per person was mostly around 20 g, but the amount of more than 60 g was occasionally reported. The median value of the amount was 25 g.

The number of *K. septempunctata* spores ingested by a patient was estimated to be over  $10^7$  per person from the reported data on the amount of ingested olive flounder and number of the spores in the olive flounder. The attack ratio would be likely to decrease with the decrease of the amount of ingested spores when the ingested number is less than  $10^7$  per person. More exact dose-response relationships remain unclear because of the lack of epidemiological observations. Furthermore, the amount of ingested olive flounder involves estimations based on interviewed data. Also various factors including the activity of the spores and susceptibility of individuals are possibly involved in onset of the symptoms. Although such uncertainties exist, ingestion of  $10^7$  spores per person is considered to elicit major symptoms including diarrhea and vomiting.

Japanese local governments investigated the origin of olive flounder implicated in 64 outbreaks which occurred in 2013 and 2014. Among the outbreaks, 44 were caused by imported cultured olive flounder, 10 were by domestic natural olive flounder, and one was by domestic cultured olive flounder. Information was not disclosed for two outbreaks, and the origin of olive flounder was unclear for 7 outbreaks. Among 57 outbreaks for which the origin of implicated olive flounder was traced back, 77% were linked to imported cultured olive flounder. Consistent with it, violations against regulation were identified in imported olive flounder by inspection of *K. septempunctata* at the import. However, only 2% of the outbreaks were associated with domestic cultured olive flounder. In June 2012,

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<sup>3</sup> DALY is a measure of overall disease burden expressed as the number of years lost due to ill-health, disability or early death. The DALY is used internationally as a comprehensive and quantitative index of disease burden, not only in the area of food safety.



the Ministry of Agriculture, Forestry and Fisheries notified to the local governments and relevant organizations to implement prevention measures in domestic fish farms. The measures included an introduction of the flounder seeds free from *K. septeempunctata* to fish farms and seed producing facilities, culture management with grouping of olive flounder of different history, and pre-shipment inspection. The prevention measures were considered to be effective based on the drastic decrease in the number of food poisoning caused by domestic cultured olive flounder since 2013.

Prevention measures against contamination with *K. septeempunctata* in olive flounder at the production stage are thus considered to be important in reduction of the risk to human health, although life cycle of and infection route to the flounders are yet unknown.

According to the provisional calculation of DALYs mentioned above, the burden of disease caused by *K. septeempunctata* is considered to be remarkably low.

Taking into account the provisional DALYs, the risk management organizations are urged to consider possible prevention measures against this parasite. Implementation of the prevention measure in fish farm is continuously crucial. In addition, under the current situation where outbreaks of *K. septeempunctata* poisoning still occur, it is important to continue watching of the trend of the food poisoning. Detailed investigations of the outbreaks are also important to identify the source of contamination, and to determine the amount of consumed olive flounder and spore concentrations in them.

Pre-shipment inspection or inspection at the import with appropriate regulatory standards fitting to risk managing purpose would be partially effective in reduction of highly contaminated olive flounder, because such inspections would give a caution to the producers and exporting countries. Low proportions of parasitized flounders and large variations of the number of the parasite in the flounders even from the same fish farm should be kept in mind at inspections of the imports. Moreover, the prevention measure in fish farms is considered to be effective for the imported cultured flounders as well as for domestic ones rather than prevention measures depending on test of the parasite contamination in imported fish at the import.