OUTBREAKS OF FOODBORNE DISEASES ASSOCIATED WITH THE FOOD TOXICITY CAUSED BY KUDOA SEPTEMPUNCTATA IN OLIVE FLOUNDER (Paralichthys olivaceus)

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SUMMARY: Kudoa septempunctata is a myxosporean parasite of Paralichthys olivaceus (olive flounder) that causes more than 50 cases of foodborne illness in Japan each year. For quantitatively assessing the presence of K. septempunctata spores in the causative fish at food poisoning outbreaks, both a direct observation method using microscopy and a quantitative real-time PCR (qRT-PCR) method are officially accepted in Japan. However, lower correlations have been often noticed between the number of spores counted using the direct observation method and the DNA amount determined using the qRT-PCR method. To elucidate the cause of this discrepancy, we observed muscle tissues of infected olive flounders with K. septempunctata by transmission electron microscopy. The images demonstrated unsynchronized development of K. septempunctata spores in plasmodia found within myofibers; in other words, the plasmodium contained not only developed spores with completed shell valves but also developing spores (sporoblasts) composed of spore-forming cells without shell valves. Furthermore, the ratio between developed spores and sporoblasts varied at different parts of muscles. The direct microscopic observation method could count developed spores, whereas the qRT-PCR method could quantify the amount of not only spores but also sporoblastic cells regardless of the cellular development and differentiation. Considering that the food toxicity caused by K. septempunctata is induced by viable spores passing through the gastric environment, the direct observation method counting only developed spores is better than the qRT-PCR method for assessing the cause of foodborne illness at the outbreak as well as the risk of human illness in monitoring surveys of aquacultured or natural-water fish.

Electron Microscopic Study of Kudoa septempunctata Infecting Paralichthys olivaceus (Olive Flounder)

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also from any other cellular structures such as sporoblasts, the proportion of sporoblasts in a plasmodium may interfere with the assumed spore numbers based on the qRT-PCR method. However, the details of *K. septempunctata* in *P. olivaceus* muscle are not well confirmed. In this study, we precisely observed *K. septempunctata* in *P. olivaceus* muscles by transmission microscopy.

In this study, *P. olivaceus* infected with *K. septempunctata* was purchased from a local fish farm in Japan and maintained at the National Research Institute of Aquaculture Fisheries Research Agency. Direct observation by optical microscopy and the qRT-PCR assay were performed as described previously (8). Transmission electron microscopy was performed as follows: the cubical muscles of *P. olivaceus* with side ca. 3 mm were fixed overnight with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (PB), pH 7.4, at 37°C, followed by post fixation with 2% osmium tetroxide in 0.1 M PB at 4°C for 2 h. The samples were dehydrated through an ethanol series (50%, 70%, 90%, and 100%). After dehydration, the samples were infiltrated with propylene oxide (PO) twice for 20 min each and added to a 70:30 mixture of PO and resin (Quetol-812; Nissin EM Co., Tokyo, Japan) for 1 h. The samples were then transferred to a fresh resin and polymerized at 60°C for 48 h. Ultra-thin sections of the blocks were prepared using an ultramicrotome.
(ULTRACUT UCT; Leica Microsystems GmbH, Wetzlar, Germany), and the sections were placed on copper grids and stained with 2% uranyl acetate at room temperature for 15 min, followed by secondary staining with lead stain solution (Sigma-Aldrich Co., St. Louis, Mo., USA) at room temperature for 3 min. The grids were observed by transmission electron microscopy (JEM-1200EX; JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 80 kV.

Transmission microscopy clearly found the plasmodia containing various Kudoa cells, forming pseudocysts, in myofibers of P. olivaceus (Fig. 1a). The plasmodial periphery demarcated a border between a K. septempunctata cellular mass and host cellular components (Fig. 1b). In the plasmodia, spores at different stages of development, including sporoblasts, were observed, indicating the unsynchronized development of K. septempunctata spores even in a single plasmodium (Fig. 1c–g). A similar observation has been reported for other Kudoa spp. (3–5). The ratio of sporoblasts to spores varied among different regions of P. olivaceus muscles. In some regions of the muscles, the number of sporoblasts was almost equivalent to that of spores (Fig. 1c–g). In contrast, there were other regions that consisted almost entirely of spores (Fig. 1a, h, and i). These results indicate that the qRT-PCR method will detect not only spores but also sporoblasts and that the ratio of sporoblasts to spores significantly influences the qRT-PCR results, leading to the low correlation between the results from direct observation and qRT-PCR. Because it is supposed that the toxicity of K. septempunctata is mainly provoked by spores (1,6,7), the direct observation method would be more reliable than the qRT-PCR method for the examination of Kudoa foodborne disease and inspection of K. septempunctata spores to prevent illness. On the other hand, the qRT-PCR method would be appropriate for the detection of K. septempunctata cells at all development stages, particularly for larva of P. olivaceus in which only sporoblasts are developed. In addition to the examination of K. septempunctata, the examination methods should be carefully selected for the quantification of parasites with similar nature to K. septempunctata, such as other Kudoa spp.

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Conflict of interest None to declare.

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