Short communication

Lymphocystis disease in cultured false clown anemonefish (Amphiprion ocellaris)

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ABSTRACT

False clown anemonefish (Amphiprion ocellaris) is one of the most famous marine ornamental fish which have a highly economic value in Thailand. The affected fish showed external lesions of irregular white nodules in various sizes on skin, fins and mouth. Histopathological finding revealed clusters of lymphocystis hypertrophied cell with thick smooth hyaline capsule. The infected cell showed an enlarged nucleus with basophilic marginated chromatin and basophilic intracytoplasmic inclusion bodies, mainly in the peripheral area of the cell. The typical cells were found in skin, fins, operculum, gill, spleen, and kidney. Transmission electron microscopy revealed an icosahedral viral particle measured 200 nm in diameter with dense nucleocapsid and a well-defined electron-lucent envelope with surface-associated fibrils. This is the first report of lymphocystis disease in cultured false clown anemonefish in Thailand.

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1. Introduction

Anemonefish is one of the ornamental marine fish which are mostly admired because of their various colors and beauty. Anemonefish are in the family; Pomacentridae, subfamily; Amphiprioninae and native to warm waters of the Indian and Pacific oceans. Clownfish live in small groups inhabiting a single anemone. Twenty eight species are recognized, one in the genus Premnas, while the remaining are in the genus Amphiprion. However, there are only 7–8 species in Thailand. Five species of anemonefish; Amphiprion ocellaris, Amphiprion akallopisos, Amphiprion clarkia, Amphiprion sebae and Amphiprion ephippium, are found in Andaman sea while the species Amphiprion polymnus and Amphiprion perideraion are found in the gulf of Thailand.

The false clown anemonefish (A. ocellaris) are tropical marine fish frequently found in Andaman sea. They inhabit coral reefs and sheltered lagoons up to a depth of 15 m. False clown anemonefish have orange to reddish-brown color with three white bands on the head, body, and caudal peduncle. The white bands are outlined in black. The outer margin of fins is white and the inner one is black. Clownfish or anemonefish culture is increasingly popular and rapidly widespread in Thailand. An expansion of anemonefish culture resulted in an increase of smuggling anemonefish fishing from the nature. The population of anemonefish became rapidly decreased and almost reached a crisis point. In order to respond to an uprising demand for anemonefish of domestic and overseas market, an organization of government and private sector is now focusing on the production of anemonefish larva population with high quantity and survival rate. However, the fish culture is difficult to avoid diseases because it only focuses on a high production with no consideration of a management. Consequently, the cultured area is very crowded and lacks appropriate management. This leads to stress and epidemic of disease easily especially infectious diseases.

Lymphocystis disease (LCD), one of the common infectious diseases which affect marine fish cultures in Thailand, was discovered in 1874 (Zhang et al., 2004). Distribution has been reported worldwide such as in Spain (Alonso et al., 2005), France (LeDuff et al., 1993), Korea, Japan (Hossain et al., 2008) and China (Sheng et al., 2007). In Thailand, the disease was reported by Wachirachaipaisal and Limsuwan in 1985. Over 125 fish species were reported including 25 marine species, belonging to 42 families (Cano et al., 2006, Sheng et al., 2007, Cano et al., 2009). The causative agent of LCD is lymphocystis disease virus (LCDV) which is a large virus in the genus Lymphocystivirus of the family Iridoviridae. LCDV is an icosahedral symmetry virus, approximately 200–300 nm in diameter, and contains single linear double stranded DNA which is circularly permuted and terminally redundant (Darai et al., 1983; Tidona and Darai, 1997; Kitamura et al., 2006). LCD is characterized by the external appearance of nodules, either singly or in groups, on skin, fins, or tail of the affected fish (Walker and Hill, 1980; Alonso et al., 2005). Although, LCD is not a fatal disease, the external appearance might cause a significant economic loss. The principle mode of transmission of LCD is horizontally via direct contact (Cano et al., 2006) and external trauma (Alonso et al., 2005). Other factors such as water contamination (Overstreet, 1988) and stress condition caused by high population density, nutrition deficiencies, decreased dissolved oxygen, suboptimal water quality, or human manipulation may increase
the appearance of LCD symptoms (Paperna et al., 1982; Mellergaard and Nielsen, 1995; Alonso et al., 2005; Cano et al., 2006). The recent study reported that *Artemia* sp. might act as a reservoir host of this disease (Cano et al., 2009). To our knowledge, LCDV infection has never been reported in cultured false clown anemonefish. The purposes of this study were to report the occurrence of LCDV in false clown anemonefish and to determine the distribution of LCDV in fish organs.

### 2. Materials and methods

#### 2.1. Fish samples

Eight naturally infected cultured false clown anemonefish (*A. ocellaris*), with an average body length about 1 inch, were collected from a cultured farm in central of Thailand. The morbidity...
rate of this farm was 20%. Fish were transported to the laboratory using a plastic bag method and then euthanized using clove oil before complete examination. The fish showed typical external signs of LCDV nodules in different sizes mainly on the body skin surface, fins, tail, and mouth. Gross pathology, histopathology, and transmission electron microscope (TEM) were used as diagnostic tools.

3. Results

3.1. Macroscopic finding

The nodular lesions of skin samples were cut into 6–8 small squares, approximately 0.5–1 mm. and then fixed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) overnight at 4°C for 24 h. The specimens were then dehydrated in ethanol and placed into xylene in order to remove ethanol from the tissues. Furthermore, they were transferred to an embedding mold of fresh parafﬁn. All parafﬁn blocks were then secured to the microtome and sectioned (4–6 μm in thickness). Finally, the ﬂatten sections were mounted onto slides and stained with hematoxylin and eosin (H&E) for histopathological study.

2.2. Pathological study

All of the fish samples were examined for gross pathology including the location, distribution, shape, size, color, consistency and special features of typical external lesions. Fish samples with lymphocystis nodules were selected and ﬁxed with 10% buffered formalin solution at room temperature for 24 h. The ﬁxed samples were rinsed offtap water and then were cut into small pieces approximately 0.5 cm in transverse section. Next, all of the specimens were decalcified with formic acid sodium citrate solution for 24 h and immersed in formalin solution at room temperature for 24 h. After that, tissues were dehydrated in ethanol and placed into xylene in order to remove ethanol from the tissues. Furthermore, they were transferred to an embedding mold of fresh parafﬁn. All parafﬁn blocks were then secured to the microtome and sectioned (4–6 μm in thickness). Finally, the ﬂatten sections were mounted onto slides and stained with hematoxylin and eosin (H&E) for histopathological study.

2.3. Electron microscopy

The nodular lesions of skin samples were cut into 6–8 small squares, approximately 0.5–1 mm and then ﬁxed in 2.5% glutaraldehyde in phosphate-buffered saline (PBS) overnight at 4°C post-ﬁxed in 1% osmium tetroxide with ferricyanide in PBS at 4°C for 1 h. The specimens were then dehydrated in an ascending ethanol series and then ﬁnally embedded in Epon epoxy resin following standard procedures. Ultrathin sections were double-stained with lead citrate and uranyl acetate for observing and photographing with a transmission electron microscope (TEM).

3. Discussion

This is the ﬁrst report demonstrating the presence of LCDV in cultured false clown anemoneﬁsh. The macroscopic ﬁnding was similar to the common characteristics of LCDV infection in ﬁsh such as infected Sarcocentrum rubrum (Wachirachaipaisal and Limsuwan, 1985) and Chanda ranga (Williams et al., 1996) which were reported in Thailand. Histopathology and electron microscopy conﬁrmed the typical lymphocystis cells containing iridovirus-like particles.

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References


