Surveillance of Epidemic Serotype of *Miamiensis avidus* Causing Scuticociliatosis in Japanese Aquariums

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ABSTRACT: cuticociliatosis is one of the most important parasitic diseases in aquaculture farms as well as aquariums. The causative agent of the disease is *Miamiensis avidus*, and there are at least three serotypes of this ciliate. Vaccines against either of two serotypes have provided no cross-protection in vaccinated fish challenged with heterologous serotypes. Therefore, in this study, we surveyed the epidemic serotypes of this parasite in Japanese aquariums for the development and use of effective vaccines. Diseased 52 fishes (26 kinds) were collected from different aquariums. *M. avidus* was detected from all fishes by species-specific PCR. After culturing the ciliates, we identified the serotype by serotype-specific PCR. From the results, 46, 0, and 1 ciliate isolates were identified as serotype I, II, and III, respectively, whereas 5 isolates were found to be of an unknown serotype. This suggests that serotype I is the epidemic serotype in Japanese aquariums. Since serotypes I and II are evenly distributed in aquaculture farms, the epidemic serotypes in aquariums and farms may be different.

INTRODUCTION

Scuticociliatosis is an important parasitic disease in global aquaculture industries, and scuticociliates have been isolated and/or detected in the diseased fishes. The disease is frequently observed in the Japanese flounder, *Paralichthys olivaceus*, one of the major fish species cultured in Asian countries such as Japan, Korea, and China. A lethal systemic scuticociliate infection has been reported in aquarium-captive zebra sharks (*Stegostoma fasciatum*), Port Jackson sharks (*Heterodontus portusjacksoni*), and Japanese horn sharks (*Heterodontus japonicus*) (Stidworthy *et al*. 2014). In other aquariums, the disease was observed in two different kinds of sea dragons (*Phycodurus eques* and *Phyllopteryx taeniolatus*) (Rossteuscher *et al*. 2008). Thus, controlling the disease is important in not only aquaculture farms, but also aquariums.

The causative agent of the disease is *Miamiensis avidus*, and there are at least three serotypes (Serotype I, II, and III) of this ciliate （Song *et al*. 2008）. The three serotypes are distinguishable by the serotype-specific antigenic polypeptides (serotype I, 30 kDa; serotype II, 38 kDa; serotype III, 34 kDa). Because there is no effective chemical treatment method for scuticociliatosis, vaccination may be the key for the prevention of the disease. However, vaccines showed beneficial effects only against infection with a homologous serotype (Piazzon *et al*. 2008). Therefore, a survey for pandemic serotypes would be important for the development of an effective vaccine.

MATERIALS AND METHODS

Scuticociliate

Scuticociliatosis naturally occurred at four geographically separated aquariums in Okinawa, Fukuoka, Chiba, and Tokyo in Japan. At necropsy, multiple macroscopic lesions, which were often hemorrhagic, and ulcerations were observed; these lesions were found to be confined to the skin. Histopathologically, epidermal ulcers were associated with necrosis and inflammation of the underlying dermis and musculature. Numerous ciliates were observed in these lesions. In several fishes, these ciliates had invaded the blood vessels and were detected in the gills and internal organs including the ovaries, epithalamus, and brain. The scales of the affected fishes were used to culture the ciliates in Eagle's minimum essential medium containing penicillin (10 unit /ml) and streptomycin (10 µg/ml) at 23°C.

Detection and serotyping of M. avidus from affected fish by PCR

Genomic DNA was automatically extracted from all 52 isolates by using the mag LEAD 6GC and Mag DEA Dx SV systems (Precision System Science Co., Ltd, Japan). Species-specific PCR was

performed by the method described by Tange et al. (2010). The primer set, Ma-F (5'-GTA ACT GAT CGA ATC TCT TCA C-3') and Ma-R (5'-TTC CCG TTC ACG CAA GCG T-3'), was used, and PCR was performed using 1 cycle at 95°C for 5min, 40 cycles at 95°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min, followed by 72°C for 5 min. Serotype-specific PCR was performed as reported previously (Motokawa et al., 2018). Three primer sets were used for the amplification: Serotype I-F (5'-CAGCAGCTACTGTTGCTAATCC-3') and Serotype I-R (5'-AGCAGTACATGCGGTAGCA C-3') for serotype I, Serotype II-F (5'-AAATGC CCTGGTACTGAAGC-3') and Serotype II-R (5'-CTGCAGCAGCTAAAGCTACAC-3') for serotype II, and Serotype III-F (5'-CGCCTTAT TAGCTCTCTTCTTAGC-3') and Serotype III-R (5'-AGCAGTACAAGCATCGGAAG-3') for serotype III. PCR was performed for 30 cycles, with each cycle consisting of treatments at 94°C for 30 sec, 65°C for 30 sec, and 72°C for 1 min. The PCR products were analyzed by 2% agarose gel electrophoresis.

RESULTS

Using species-specific PCR, *M. avidus* was detected from the 52 diseased fishes (26 kinds) collected from different Japanese aquariums. The results of serotyping obtained after performing serotype-specific PCR are shown in Table 1. Among the 52 isolates, 47 isolates belonged to serotype I. Serotype II was not detected in any of the fishes, whereas serotype III was detected in 2 fishes. One *Chromis viridis* sample showed co-infection with serotypes I and III. Five isolates from four *Chromis notatus* samples and one *Ostorhinchus holotaenia* sample were not classified into any serotypes.

DISCUSSION

This is the first trial involving a massive surveillance of fish parasitic diseases in Japanese aquariums. We isolated scuticociliates from many different fish species at geographically separated aquariums in Japan. All isolates were identified as *M. avidus* by species-specific PCR and the analysis of the SSU rRNA sequences. The disease is recorded even in the Okinawa Churaumi Aquarium, which is located at a subtropical area where the seawater temperature is around 20–30°C. Because the ciliate could not grow at temperatures greater than 27°C (unpublished data), the disease may hardly occur in such regions. However, an outbreak of the disease in the aquarium was observed among deep-sea fishes reared at low water temperatures of around 16–20°C. From the results, we revealed that scuticociliatosis caused by *M. avidus* is an important infectious disease among Japanese aquariums.

Most of the *M. avidus* isolates from aquariums were identified as serotype I based on the results of serotype-specific PCR. On the contrary, the serotype II was not detected in any of the fishes. Interestingly, one *C. viridis* sample was found to be co-infected with serotypes I and III. This is the first report of co-infection with different serotypes of *M. avidus* in a single fish; however, different serotypes were detected from Japanese flounders in the same region (Motokawa et al., 2018). Since serotypes I and II are evenly distributed in aquaculture farms (Motokawa et al., 2018), the epidemic serotype in aquariums and farms may be different. Additionally, in the present study, five isolates were not classified into any serotypes. The reason for this is unclear; however, these may represent new serotype(s) because 5 genotypes of *M. avidus* were reported from the results of mitochondrial cytochrome *c* oxidase subunit 1 gene analysis (Jung et al., 2010). Further investigation regarding the appearance of new serotypes of the ciliate is needed.

One method of controlling scuticociliatosis would be cleaning the aquariums, because *M. avidus* is a free-living ciliate. However, cleaning is difficult due to the structure and pipes of the tanks (ex. pressure tank) used to house deep-sea fishes. Therefore, vaccination is an alternative for aquarium cleaning for the prevention of the disease. The serotype-specific vaccine against *M. avidus* was reportedly effective in the cultured fishes, i.e. Japanese flounders and Spanish turbots (*Scophthalmus maximus*) (Piazzon et al. 2008, publication number in international patent: WO2010044451A1). Because *M. avidus* is easily cultured in fish cell lines (Narasaki et al., 2018), it may be possible to manufacture formalin-killed vaccines in aquariums. In this study, we found that the epidemic serotype of *M. avidus* in Japanese aquariums is serotype I. Therefore, a single vaccine against serotype I is enough for prevention of the disease. In the near future, we will evaluate the effect of the vaccine for the prevention of *M. avidus* infections in aquariums.

Table 1. Serotypes of Miamiensis avidus isolates used in this study

Host fish	Fish captive location	Year isolated	Serotype PCR
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2016	I
Japanese snapper (*Paracaesio caerulea*)	Okinawa	2016	I
Longfinned bullseye (*Cookeolus japonicus*)	Okinawa	2016	I
Blue green damselfish (*Chromis viridis*)	Okinawa	2016	ND*
- (*Odontanthias katayamai*)	Okinawa	2017	I
Blue green damselfish (*Chromis viridis*)	Okinawa	2017	ND*
- (*Trichiurus sp.*)	Okinawa	2017	I
- (*Trichiurus sp.*)	Okinawa	2017	I
Blue green damselfish (*Chromis viridis*)	Okinawa	2017	ND*
- (*Trichiurus sp.*)	Okinawa	2017	I
Goldflag jobfish (*Pristipomoides auricilla*)	Okinawa	2017	I
- (*Liopropoma aragai*)	Okinawa	2017	I
- (*Plectranthias kamii*)	Okinawa	2017	I
Blue green damselfish (*Chromis viridis*)	Okinawa	2017	ND*
- (*Trichiurus sp.*)	Okinawa	2017	I
Golden threadfin bream (*Nemipterus virgatus*)	Okinawa	2017	I
- (*Trichiurus sp.*)	Okinawa	2017	I
- (*Trichiurus sp.*)	Okinawa	2017	I
- (*Trichiurus sp.*)	Okinawa	2017	I
Lavender jobfish (*Pristipomoides sieboldii*)	Okinawa	2017	I
- (*Trichiurus sp.*)	Okinawa	2017	I
Lavender jobfish (*Pristipomoides sieboldii*)	Okinawa	2017	I
Japanese chromis (*Chromis mirationis*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Fryingpan snapper (*Argyrops bleekeri*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Ornate jobfish (*Pristipomoides argyrogrammicus*)	Okinawa	2017	I
Japanese snapper (*Paracaesio caerulea*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Japanese snapper (*Paracaesio caerulea*)	Okinawa	2017	I
Ornate jobfish (*Pristipomoides argyrogrammicus*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Ornate jobfish (*Pristipomoides argyrogrammicus*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Rosy dwarf monocle bream (*Parascolopsis eriomma*)	Okinawa	2017	I
Threetooth puffer (*Triodon macropterus*)	Okinawa	2017	I
Yellowtail blue snapper (*Paracaesio xanthura*)	Okinawa	2017	I
Bight redfish (*Centroberyx druzhinini*)	Okinawa	2017	I
Blue green damselfish (*Chromis viridis*)	Fukuoka	2017	III
Green eel goby (*Odontamblyopus lacepedii*)	Fukuoka	2017	I
Redlip mullet (*Chelon haematocheilus*)	Fukuoka	2017	I
Rocksucker (*Chorisochismus dentex*)	Tokyo	2018	I
Copperstriped cardinalfish (*Ostorhinchus holotaenia*)	Tokyo	2018	ND*
Pigmy sweeper (*Parapriacanthus ransonneti*)	Tokyo	2018	I
Japanese butterflyfish (*Chaetodon nippon*)	Tokyo	2018	I
Blue green damselfish (*Chromis viridis*)	Chiba	2018	I
Blue green damselfish (*Chromis viridis*)	Chiba	2018	I&III
Blue green damselfish (*Chromis viridis*)	Chiba	2018	I
Japanese whiptail (*Pentapodus nagasakiensis*)	Okinawa	2018	I
Japanese snapper (*Paracaesio caerulea*)	Okinawa	2018	I
Checked swallowtail (*Odontanthias borbonius*)	Okinawa	2018	I
Japanese chromis (*Chromis mirationis*)	Okinawa	2018	I
Okinawa chromis (*Chromis okamurai*)	Okinawa	2018	I
Black scraper (Thamnaconus modestus)	Tokyo	2018	I
Ornate jobfish (*Pristipomoides argyrogrammicus*)	Okinawa	2018	I
Ornate jobfish (*Pristipomoides argyrogrammicus*)	Okinawa	2018	I

*Not determined

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