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Mass mortalities associated with viral nervous necrosis in hatchery-reared groupers in the People's Republic of China

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ABSTRACT—The viral etiology of mass mortalities of groupers, *Epinephelus coioides* and *E. akaara*, cultured in the People's Republic of China was examined. Disease outbreaks occurred in 7 to 45 day-old fish with erratic swimming motion and marked vacuolation was observed in the brain and retina of the affected fish. The piscine nodavirus (the Betanodavirus), the causative agent of viral nervous necrosis (VNN), was detected in the affected tissues by electron microscopy, indirect fluorescent antibody test and reverse transcription-polymerase chain reaction. This paper is the first record of the agent in the People's Republic of China.

Key words: betanodavirus, viral nervous necrosis, VNN, *Epinephelus coioides*, *Epinephelus akaara*, China

Rapid development of marine fish culture in the world has been accompanied by emerging infectious viral diseases. Among these viral diseases, viral nervous necrosis (VNN) caused by betanodaviruses (piscine nodaviruses) is one of the most devastating diseases in a variety of cultured marine fish[1]. VNN has spread over Asia, Oceania, Europe and North America for this decade[1, 2]. The affected fish is characterized by abnormal swimming behavior and vacuolation in the central nervous tissues. Round-shaped, non-enveloped viral particles (25 to 30 nm in diameter) are observed in the cytoplasm of affected retinal and brain cells.

The present paper describes VNN of orange-spotted grouper (*Epinephelus coioides*) and red-spotted grouper (*E. akaara*) occurred at the Guangdong Daya Wan Fisheries Development Center (GDFDC), located in the southern China, which is one of grouper seed production centers in the People's Republic of China. This is the first case report of VNN in the People's Republic of China.

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Materials and Methods

At the GDFDC, broodfish of orange-spotted grouper (3–7 years old) and red-spotted grouper (3–8 years old) spawned naturally in 75-m^3 tanks, and fertilized eggs were collected by an overflow system and transferred into 10 to 45-m^3 tanks. Hatched larvae were reared at a density of 30,000 fish per m^3, and larvae and juveniles were fed with rotifer, *Artemia* nauplii, and minced fish according to their developmental stages.

Moribund fish (9–15 mm in total length) were fixed with 10% buffered formalin and embedded in paraffin wax. Sections of 5 μm were stained with haematoxylin and eosin for histopathological examinations or subjected to an immunofluorescence staining with a rabbit antiserum against SJNNV (striped jack nervous necrosis virus)[3]. For transmission electron microscopy, red-spotted grouper samples were fixed in a 2.5% glutaraldehyde- 2% paraformaldehyde mixture, post-fixed in 1% osmium tetroxide and embedded in Quetol 812. Sections were examined under a transmission electron microscope (Hitachi H-7500).

Total RNA was extracted from diseased fish using ISOGEN (Nippon gene) according to the manufacturer's direction. Reverse transcription-polymerase chain reaction (RT-PCR) was carried out to detect the coat protein gene of betanodavirus with two primers (F2, R3) by the method described previously[4]. These PCR primers were designed for amplifying RNA2 T4 region (426 bp) of SJNNV. Amplified DNA was analyzed by 2% Nusieve-agarose (3 : 1) gel electrophoresis.

Results and Discussion

The GDFDC started the seed production programme for orange-spotted grouper and red-spotted grouper in 1997. Although mortalities (approximately 90%) with clinical signs similar to VNN occurred in larval and juvenile red-spotted grouper but not in orange-spotted grouper in 1999, the viral aetiology of mortalities remained to be solved. During June to July in 2000, mass mortalities reaching nearly 100% were encountered again in larvae and juveniles of both fish species. The disease occurred in red-spotted grouper of 9–25 mm in total length (about 20–45 days post-hatching) and in orange-spotted grouper of 3–25 mm (about 7–40 days). Affected fish showed abnormal swimming behavior such as whirling near the water surface, abrupt corkscrew-like swimming, sinking

to the bottom and then floating to the water surface. Some diseased fish were dark colored. No severe mortalities occurred in juveniles larger than about 25 mm in both species. The water temperature when mass mortalities occurred was 25 to 30°C.

Under light microscope, heavy vacuolation was observed in the brain and retina of affected larvae and juveniles of both orange-spotted and red-spotted groupers (Fig. 1), though the extent of lesions varied considerably from fish to fish. The vacuolated tissues were strongly positive for immunofluorescence test. All other organs examined showed no conspicuous histopathological changes. Electron microscopy revealed that myriad of nonenveloped, spherical virus particles (25–28 nm in diameter), were observed in paracrystalline arrays in the cytoplasm of degenerated nerve cells (Fig. 2). In PCR test, a 430 bp-amplicon was constantly detected from nucleic acid preparations of both fish species (Fig. 3). Furthermore, the virus was isolated from eyes and brains of diseased fish using E-11 cell line[5] (data not shown). These results indicate that the present mass mortalities of orange-spotted and red-spotted groupers at GDFDC in 2000 were associated with VNN.

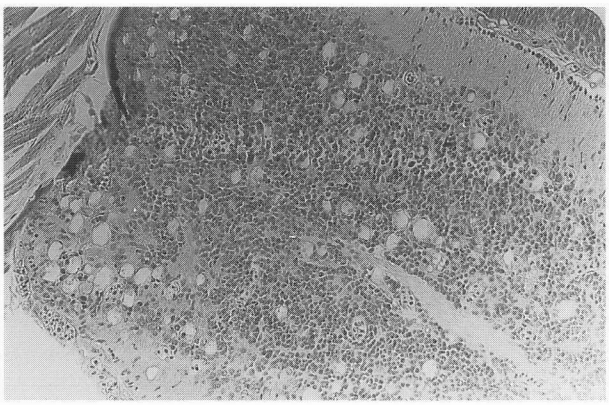

Fig. 1. Light micrograph showing vacuolation in the brain of orange-spotted grouper.

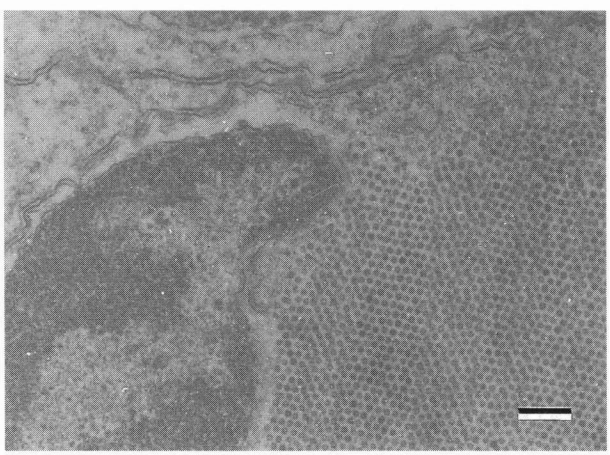

Fig. 2. Virus particles in the cytoplasm of a retinal cell of red-spotted grouper. Bar = 200 nm.

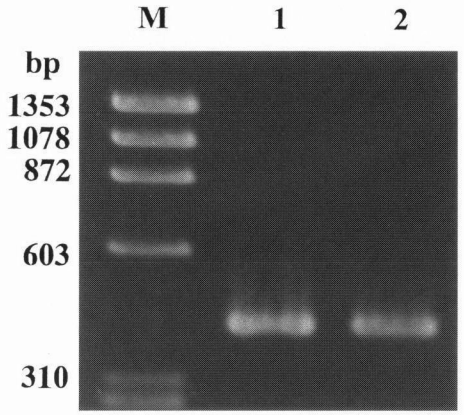

Fig. 3. Agarose gel electrophoresis of PCR products from diseased fish. Lanes M: DNA marker, 1: diseased orange-spotted grouper, 2: red-spotted grouper.

Twenty or more marine fish species have been reported as natural hosts of betanodaviruses[1, 6]. VNN in the family *Serranidae* was first described in red-spotted grouper *E. akaara*[7], and thereafter other 6 host species, *E. moara, E. septemfasciatus, E. malabaricus, E. tauvina, E. fuscogutatus,* and *Cromileptes altivelis* were reported in Asian countries[8–13]. *E. coioides* in our study is a new host species of betanodavirus, and this paper is the first record on VNN in the People's Republic of China.

Interestingly, it has been revealed that betanodavirus isolates from groupers belong to the same RGNNV genotype and this genotype virus proliferates well at higher temperature in cultured cells compared with other 3 genotypes (SJNNV, TPNNV, BFNNV), indicating warm-water fish origin of this genotypic variant[5]. Another finding on VNN of groupers is that the infection often occurs even at grow-out stages[9]. In our study, however, mortalities naturally decreased when fish reached approximately 25 mm in total length, suggesting that the present causal betanodavirus might differ from the previous groupers' ones. Further investigations will be required to reveal genotypic and phenotypic features of the present betanodavirus isolates. It is a matter of urgency how we prevent further spread of the disease in People's Republic of China.

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