

PCR Detection of Piscine Nodaviruses in Several Fishes and Invertebrates in Korean Peninsula

D.K. Gomez^{1, 4}, S. C. Park^{2, 4}, G.W. Baeck³ and J. H. Kim²

¹College of Fisheries and Aquatic Sciences, Iloilo State College of Fisheries, Iloilo, Philippines

² College of Veterinary Medicine, Seoul National University

³Department of Oceanography, Pukyong National University

⁴ KRF Zoonotic Disease Priority Research Institute, Seoul National University

INTRODUCTION

Viral nervous necrosis (VNN) or viral encephalopathy and retinopathy (VER) caused by piscine nodavirus (*Nodaviridae*, *Betanodavirus*) has distributed worldwide for these 2 decades in cultured populations of marine fish. The present study was conducted to investigate asymptomatic infection of piscine nodavirus in several fish and invertebrate populations.

MATERIALS AND METHODS

Apparently healthy cultured flounder *Paralichthys olivaceus* (63 samples), were collected in flounder culture facility in Jeju Island. While apparently healthy cultured marine fish (33 samples, 7 species) were collected in the fish culture facility in Namhae area. Apparently healthy wild marine fish (663 samples), consisting of 78 species were collected near the culture facilities in three different coastal areas [Donghae (East), Hwanghae (West), Namhae (South)] of Korean Peninsula. A total of 36 samples (5 species) of apparently healthy freshwater fish were also collected in the river of Hwanghae (West) area. The RNA of the brain of fish was extracted and examined by reverse transcriptase polymerase chain reaction (RT-PCR) and nested PCR assays.

RESULTS AND DISCUSSION

In the flounder culture facility at Jeju Island, all samples were negative for nodavirus in both RT-PCR and nested PCR tests. While the marine fish in the culture facility of Namhae area, 2 samples were positive for nodavirus in nested PCR assay. In

Donghae (East) areas, 14 wild marine fish were negative for nodavirus in both RT-PCR and nested PCR tests. In Hwanghae (West) areas, 80 wild marine and 36 wild freshwater fish were all negative for nodavirus in both RT-PCR and nested PCR tests. While in Namhae (South) area, 48 of 569 wild marine fish were positive only for nodavirus in nested PCR test. The detection rate in nested PCR is $50/795 = 6.3\%$. On the other hand, One hundred samples (20 species) of wild marine invertebrates were also collected from western (Hwanghae) and southern (Namhae) part of Korean Peninsula. The RNA of the brain or other organs of the invertebrates were also extracted and examined for nodavirus by RT-PCR and nested PCR tests. In 40 wild marine invertebrate samples (5 species) collected at Hwanghae (West) areas, all samples were negative for nodavirus in both RT-PCR and nested PCR tests. Of the 60 wild marine invertebrates (12 species) collected at Namhae (South) area, 4 invertebrate samples were positive only for nodavirus in nested PCR test. Positive nested PCR results were obtained in the brain of crab species, *Charybdis bimaculata* Charybdid crab hepatopancreas of shrimp species, *Pandalus hypsinotus* Southern humpback shrimp and pooled organs of mussel species, *Mytilus galloprovincialis* Mediterranean mussel. The detection rate in nested PCR is $4/100 = 4\%$.

These results illustrate that some populations of wild and ornamental marine fish and invertebrates around the Korean Peninsula are inapparently infected with piscine nodavirus, suggesting an importance of these asymptomatic carrier fish or invertebrates as natural hosts or reservoir of betanodavirus.

REFERENCES

- Gomez D. K., J. Sato, K. Mushiake, T. Isshiki, Y. Okinaka, and T. Nakai (2004): PCR-based detection of betanodaviruses from cultured and wild marine fish with no clinical signs. *J. Fish Dis.*, 27, 603-608.
- Sri Widada J., S. Durand, I. Cambournac, D. Qian, Z. Shi, E. Dejonghe, V. Richard and J. R. Bonami (2003): Genome-based detection methods of *Macrobrachium rosenbergii* nodavirus, a pathogen of the giant freshwater prawn, *Macrobrachium rosenbergii*: dot-blot, in situ hybridization and RT-PCR. *J. Fish Dis.*, 26, 583-590.