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Short communication

Survey of viral and bacterial pathogens in ornamental aquatic crustaceans imported into South Korea

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Cherry shrimp (*Neocaridina davidi*)

ABSTRACT

The annual rate of ornamental aquatic crustacean importation is increasing globally. The continuous high demand for live ornamental aquatic crustaceans in many countries has also increased the frequency of imported exotic pathogens, which has caused many problems. In the present study, we examined the monthly distribution of viral and bacterial pathogen genes in ornamental aquatic crustaceans imported into South Korea during a 6-month period from February to July 2016. Among the bacterial pathogens identified, *Vibrio alginolyticus* was detected in cleaner shrimp in June, and *Photobacterium damselae* was detected in cleaner shrimp in July. *Vibrio penaeicida* was detected in peppermint shrimp, banded coral shrimp, hinge-beak shrimp, and orange swamp crayfish between February and June. Among the viral pathogens identified, spawner mortality virus was detected in cherry shrimp in May. Finally, hepatopancreatic parvovirus was detected in both cherry shrimp and orange swamp crayfish between February and March. Notably, the genes of two viral pathogens were also detected in cherry shrimp. Although the viruses were not isolated, they are very likely to have been introduced into South Korea and have an influence on Korean aquaculture. In South Korea, HPV is already prevalent and has been reported several times in aquaculture and in the wild; however, SMV infection has not been reported to date. These results demonstrate that many bacterial and viral pathogens are regularly introduced into South Korea via the ornamental aquatic-crustacean trade.

1. Introduction

The annual import and export of ornamental aquatic crustaceans are increasing globally. Moreover, the import of live crustaceans from South Korea shows a continuous increasing trend by approximately 0.2% every year based on data from the Korea Maritime Fisheries Development Institute. According to data from the K-Fish Information portal, from 2013 to 2016, on average, South Korea imported ornamental aquatic species worth $1,286,000 from Thailand, $828,000 from Indonesia, $629,000 from Singapore, $572,000 from China, $373,000 from the United States, $281,000 from Malaysia, and $215,000 from Taiwan. In the past few decades, the outbreak of infectious diseases in aquaculture has been likely due to influx of pathogens from abroad. Therefore, quarantining imported aquatic species is important to prevent the outbreak of increasing disease.

The major crustacean species in Korean aquaculture is the Pacific white shrimp (*Litopenaeus vannamei*). The white spot syndrome virus (WSSV) is a common viral disease in prawns, and the occurrence of this disease has increased over the last 20 years. A new disease emerging in prawn farming is acute hepatopancreatic necrosis disease (AHPND), which has been reported to rapidly increase the mortality to 100% and is not characterized by any symptoms. It has spread to many countries over the past decade, resulting in many billions of dollars of loss to the prawn aquaculture industry. The first report of outbreaks resulting in significant production losses was from Hainan, Southern China, in 2009. In addition, disease occurrences have expanded to fry prawn farms in Guangdong, Fujian, and Guangxi in China, resulting in approximately 80% losses in production during the first half of 2011. In Vietnam, AHPND was reported for the first time in 2010, and it continues to affect farms. In Malaysia, this viral infection was first reported on the east coast in 2010, subsequently causing estimated reported losses of $100 million in Pahang, Perak, and Penang in 2011. Thailand

Abbreviations: aa, amino acid; AHPND, acute hepatopancreatic necrosis disease; GAV, gill-associated virus; HPV, hepatopancreatic parvovirus; MBV, *Penaeus monodon* baculovirus; MCMS, mid-crop mortality syndrome; MSGS, monodon slow growth syndrome; NCBI, National Center for Biotechnology Information; NHP, necrotizing hepatopancreatitis; PCR, polymerase chain reaction; SMV, spawner mortality virus; TBV, tetrahedral baculovirosis; TCBS, thiosulfate-citrate-bile salts-sucrose

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reported mass mortalities in shrimp farms in the eastern coastal province due to AHPND in 2012. AHPND has also expanded to a wider global area. In Mexico, because of AHPND infection, prawn production decreased by approximately 80% in 2013. As the number of crustaceans that are imported and exported worldwide increases, the incidence and infection area of diseases also increase; thus, continuous evaluation of imported aquaculture species is urgently needed (OIE, 2014, 2017).

WSSV in South Korea already has prevalent of the spread, and AHPND has spread widely in shrimp farms abroad. Furthermore, despite the international spread of these epidemics and the large amount of imported ornamental aquatic crustaceans in South Korea, transmission of the diseases related to these species has received little attention. The South Korean government has performed surveys of the non-quarantine infectious pathogens associated with imported ornamental aquatic crustaceans for the first time in the first half of 2016. In this study, we addressed this issue by measuring the presence of non-quarantine bacterial and viral pathogens in imported ornamental aquatic crustaceans.

2. Materials and methods

2.1. Sampling of imported ornamental aquatic crustaceans

We examined ornamental aquatic crustaceans imported into South Korea over 6 months from February to July in 2016 to detect the presence of bacterial and viral pathogens in 5–20 individuals of different species over various sampling periods. The crustaceans examined were hinge-beak shrimp (*Rhynchocinetes durbanensis*) and blue swamp crayfish (*Procambarus clarkii*) ($n = 10$ per month for 4 months from April to July and 3 months from May to July), banded coral shrimp (*Stenopus hispidus*) ($n = 5$ and 10 per month in February–March and April–June, respectively), orange swamp crayfish (*Procambarus clarkii*) and cherry shrimp (*Neocaridina davidi*) ($n = 10$ and 15 per month in February–March, respectively, and 20 per month thereafter), peppermint shrimp (*Lysmata wurdemanni*) and cleaner shrimp (*Lysmata amboinensis*) ($n = 10$ per month in February–March and in June–July with 2 months of sampling for each species), and amano shrimp (*Caridina multidentata*) (n = 10 per month in all months) (Table 1). The different number of samples for each species was due to the irregular import of these aquatic crustaceans into South Korea.

2.2. Selection of infectious pathogens among imported ornamental aquatic crustaceans

Infectious pathogens and imported crustaceans were selected based on data from international organizations such as the World Organisation for Animal Health and OIE and survey data on aquatic species imported into South Korea over 4 years (2012–2015) from the National Fishery Products Quality Management Service of South Korea.

Therefore, we selected infectious pathogens in crustaceans for which there is an international standard method established to determine the presence or absence. The selected pathogens included bacterial pathogens such as vibriosis-causing species (*Vibrio alginolyticus*, *Vibrio penaeicida*, *Vibrio nigripulchritudo*, *Photobacterium damselae*, and *Vibrio parahaemolyticus* (acute hepatopancreatic necrosis disease, AHPND)) and pathogens causing necrotizing hepatopancreatitis (NHP) (Table 2A) and viral pathogens such as spawner mortality virus (SMV), hepatopancreatic parvovirus (HPV), pathogens causing monodon slow growth syndrome (MSGS), *Penaeus monodon* baculovirus (MBV), tetrahedral baculovirosis (TBV), and gill-associated virus (GAV) (Table 2B).

The kidney tissue from olive flounder (*Paralichthys olivaceus*) infected with *Vibrio anguillarum* or viral hemorrhagic septicemia virus was used as a positive control at each time point for pathogen detection.

2.3. Processing of imported crustaceans and detection of infectious pathogens

We asked importers to receive samples regularly and collect data to submit at the National Fishery Products Quality Management Service Institute of South Korea for the detection of bacterial and viral pathogens in imported live crustaceans. The imported samples that were analyzed had not been exposed to a domestic environment, and all individuals were anesthetized before harvesting the hepatopancreas and muscle tissues using sterilized dissection tools.

Plates containing thiosulfate-citrate-bile salts-sucrose (TCBS) agar as a *Vibrio* species-selective medium were used to screen colonies as the first step in identification. Therefore, *Vibrio* species in the harvested tissues grew as yellow and green colonies. The grown colonies were classified by a polymerase chain reaction (PCR) assay to identify subspecies of *Vibrio* spp. We also screened for viral pathogens (SMV, HPV, MSGS, MBV, TBV, and GAV) using PCR assay. The samples for detection of virus gene were pooled, and the target organs were divided into two or four groups (5 crustaceans/group). The total DNA was extracted using the GeneAll Exgene Tissue SV kit (GeneAll Biotechnology, Korea) to use as templates in PCR assays.

2.4. PCR assay

The primer sequences used for detection of genes of each type of pathogen are shown in Table 2. The PCR methodology for pathogen detection was based on the methods described in the references listed in Table 2.

PCRs were performed in a total of 20 μL containing 10 μL of ExPrime Taq™ Premix (GENET BIO, Korea), 0.5 μM of each primer, 1 μL of 10^{-1} diluted total DNA, and distilled water. The amplification procedure included one cycle of 3 min (purified DNA) or 10 min (colony on agar medium) at 95 °C; 30 cycles of 30 s at 95 °C; 1 min at 62 °C (TBV), 60 °C (for *V. alginolyticus*, *P. damselae*, and MBV), 58 °C (for *V. penaeicida* and SMV), 55 °C (for HPV and MSGS), or 52 °C (for GAV); and 30 s at 72 °C, with a final extension step of 7 min at 72 °C. PCR products were sequenced by Cosmo Genetech (Seoul, Korea) and confirmed by BLAST searches on the National Center for Biotechnology Information (NCBI)

Table 1
Number of imported ornamental aquatic crustaceans sampled per month.

Common name (scientific name)	Total samples per month (2016)[a]						Average length (cm)	Average weight (g)
	February	March	April	May	June	July		
Peppermint shrimp (*Lysmata wurdemanni*)	10	10	0	0	0	0	3.4	0.31
Banded coral shrimp (*Stenopus hispidus*)	5	5	10	10	10	0	8.2	4.90
Hinge-beak shrimp (*Rhynchocinetes durbanensis*)	0	0	10	10	10	10	3.0	0.24
Orange swamp crayfish (*Procambarus clarkii*)	10	10	20	20	20	20	6.5	3.57
Blue swamp crayfish (*Procambarus clarkii*)	0	0	0	10	10	10	7.3	2.0
Cleaner shrimp (*Lysmata amboinensis*)	0	0	0	0	10	10	3.2	0.23
Amano shrimp (*Caridina multidentata*)	10	10	10	10	10	10	3.4	0.34
Cherry shrimp (*Neocaridina davidi*)	15	15	20	20	20	20	2.0	0.09

[a] Values represent the monthly sampling numbers for each species collected from February to July 2016.