Status of Transboundary Diseases of Penaeid Shrimps in Singapore

Wong Yelin[1]* and Jiang Junhui[2]

[1]Agri Establishment Regulation Group, Surveillance and Inspection Section, Animal Section, AVA, 6 Perahu Road, Singapore
**wong_yelin@ava.gov.sg*
[2] Technology and Industry Development Group, Aquaculture Technology Department, Marine Aquaculture Centre, AVA, St. John Island, Singapore

Abstract

Singapore has a small shrimp farming industry with approximately 54 metric tons (MT) of shrimps produced in one year from both land and coastal farms. There is also one shrimp farm producing broodstock for export. Singapore has the capability to diagnose acute hepatopancreatic necrosis disease (AHPND) through histopathology and the isolation of its causal agent which is *Vibrio parahaemolyticus*. The polymerase chain reaction (PCR) method for AHPND detection in shrimp is currently being undertaken to further strengthen its laboratory and diagnostic capacity. Notably, Singapore is still AHPND-free. On the contrary, white spot disease caused by white spot syndrome virus (WSSV) is a disease of concern as it affects the trade for ornamental crustaceans. Singapore has an active surveillance program for WSSV and other transboundary pathogens of penaeid shrimps. Positive detections would be followed by movement controls and stamping out protocols.

Introduction

Singapore is a small island state with a total population of over 5 million in 2014 (Yearbook of Statistics Singapore, 2015). With small farming land and limited fishing grounds, it imports over 90% of food consumed in the country (Agri-Food and Veterinary Authority of Singapore, 2015). Local farms have little contribution to food supply. For example, out of the 100,000 metric tons (MT) per year of fish consumed, only 5% has been attributed to domestic food fish aquaculture (Tan, 2015). Majority of the fish produced comes from coastal farming, i.e. in floating net cages, along the northern coast of Singapore. Sea bass, pompano, groupers, mullets, and milkfish are the marine food fish species popularly cultured in the country. There are also a few land-based fish farms culturing tilapia, marble goby and snakehead (Heng, 2015). The country is also known as the ornamental fish capital of the world. It exports about 2.4 million pieces of koi annually to the United Kingdom, Germany, United States and Malaysia (Ling *et al.*, 2005). Farming of penaeid shrimps in Singapore started in the 1980s with the dominance of black tiger (*Penaeus monodon*) and banana (*P. merguiensis*) shrimps. Cultivation of specific-pathogen-free (SPF) *P. stylirostris* and *P. vannamei* started in 2001 and 2002, respectively. Shrimp production reached 60 MT in early 2000 and increased to 114 MT in 2001 and 115 MT in 2002. However, due to urbanization and occurrence of diseases, shrimp production dropped to 48 MT and 46 MT in 2003 and 2004, respectively (Choi and Serena, 2005). Currently, Singapore has a small shrimp farming industry, with approximately 54 MT of shrimps produced in one year, from both land-based and coastal farms. There is also one shrimp broodstock farm producing broodstock for export.

The increase in international trade of ornamental fish poses challenge to Singapore in terms of its vigilance against exotic aquatic animal diseases (Ling *et al.*, 2005). Furthermore,

reliance on imported seafood opens the risk for transboundary aquatic animal diseases. The establishment of the Agri-Food and Veterinary Authority (AVA) has over the past years addressed these challenges and issues. AVA is the national authority for aquaculture development in Singapore and manages aquaculture farms through the issuance of fish farming licenses (Heng, 2015). It also carries out surveillance for significant pathogens and conduct regular surveys and inspections of fish farms and exporters' premises (Ling *et al.*, 2005). AVA consists of five Departments. Aquatic animal health diagnostic services are provided by one of the Departments – the Animal Health Laboratory Department (AHLD).

Acute hepatopancreatic necrosis disease (AHPND) has recently gained remarkable attention among major shrimp producing countries in Asia. It has significantly impacted the shrimp industry of China, Viet Nam, Malaysia, Thailand and the Philippines (Tran *et al.*, 2013; Joshi *et al.*, 2014; Dabu *et al.*, 2015; de la Peña *et al.*, 2015). AHPND has also been implicated in serious economic losses of cultured penaeids in Mexico (Nunan *et al.*, 2014). There is one shrimp broodstock farm in Singapore, producing broodstock for export. The farm's shrimp health monitoring programme currently in place includes samples being submitted every week to test for EMS/AHPND. Fortunately, AHPND in cultivated shrimp has not been detected yet in Singapore. On the contrary, white spot disease (WSD) caused by white spot syndrome virus (WSSV) has, over the past several years, been a disease of economic importance to Singapore as it affects the export trade of ornamental crustaceans. WSSV is also a disease notifiable to the national government of Singapore.

There is an active surveillance programme for WSSV. Other than inspections for clinical signs, samples are collected from both imported and local ornamental crustaceans for real-time PCR testing. For shrimps showing clinical signs, histopathology would also be done. Positive detections would warrant movement controls to be imposed, while further investigations would be carried out to determine the source of infection. Stamping out of the infected batch, together with other in-contact susceptible species, would be conducted. The farm would have to undergo cleaning and disinfection of the tanks/ponds before being allowed to restock. Further testing may be conducted to ascertain that the disease has been eradicated.

Status of persistent and emerging diseases of cultured shrimp

The shrimp industry in Singapore incurred significant losses in the 1990s due to outbreaks of diseases caused by yellow head virus (YHV) and white spot syndrome virus (WSSV) (Choi and Serena, 2005). Based on the annual report of AVA (2009/2010 and 2013/2014) and Ornamental Fish Newsletter (Agri-Food and Veterinary Authority of Singapore, 2010), WSSV was detected in prawns from local land-based farms and in a batch of crayfish submitted by a research institute. Over the past three years, WSSV has been detected in imported shrimps (June 2013), imported lobsters (December 2013), and imported ornamental crayfish (December 2014 and September 2015).

Laboratory and diagnostic capacity

The Animal Health Laboratory Services of AVA aims to maintain Singapore's animal disease–free status in order to facilitate the country's international trade, protect the health of the local animal populations, and indirectly safeguard human health (Agri-Food and Veterinary Authority of Singapore, 2016). It is divided into four sections namely: (a) Aquatic Animal Health, (b) Bacteriology, (c) Virology and (d) Veterinary Pathology. The laboratory was awarded with ISO/IEC 17025 SAC SINGLAS Accreditation in 2004 with more than 108 tests accredited. The Aquatic Animal Health section deals with all aquatic animal diseases. Currently, it has three veterinarians, three scientists and two laboratory technicians. In terms of training, the institute sent two veterinarians for a short shrimp pathology course at the University of Arizona, USA. AVA performs diagnostic services such as post-mortem examination, wet mount microscopy, histopathology, bacterial and fungal culture, virus isolation and molecular detections of pathogens. The section also conducts services like pre-export testing of ornamental fish, basic health monitoring of aquaculture establishments,

Table 1. List of shrimp pathogens and diagnostic methods employed at the Animal Health Laboratory Services of Agri-Food and Veterinary Authority (AVA) of Singapore.

Shrimp Pathogen	Sample Type	Diagnostic Method
Taura syndrome virus (TSV)	Pleopod or whole postlarvae (PL)	Histopathology; Real-time PCR
White spot syndrome virus (WSSV)	Pleopod or whole PL	Histopathology; Real-time PCR
Yellow head disease virus (YHDV)	Pleopod or whole PL	Histopathology; Real-time PCR
Infectious hypodermal and haematopoietic necrosis virus (IHHNV)	Pleopod or whole PL	Histopathology; Real-time PCR
Baculovirus penaei (BP)	Whole PL or shrimp	Wet mount examination; Histopathology; Real-time PCR
MBV - Spherical baculovirus (*Penaeus monodon*-type baculovirus)	Whole PL or shrimp	Wet mount examination; Histopathology
Infectious myonecrosis virus (IMNV)	Whole PL or shrimp	Histopathology; Real-time PCR
Obligate intracellular rickettsial-like organism causing necrotising hepatopancreatitis (NHP)	Whole shrimp	Histopathology; Real-time PCR
Vibrio parahaemolyticus causing acute hepatopancreatic necrosis disease (VP_{AHPND})	Whole shrimp or hepatopancreas	Culture and biochemical tests for *Vibrio parahaemolyticus* (VP_{AHPND}); Histopathology
Enterocytozoon hepatopenaei (EHP)	Whole shrimp or hepatopancreas	Histopathology

Table 2. List of molecular methods, equipment, and primers used for the detection of infectious shrimp pathogens at the Animal Health Laboratory Services of Agri-Food and Veterinary Authority (AVA) of Singapore.

Shrimp Pathogen	Method, equipment, and primers used
Taura syndrome virus (TSV)	TaqMan Real Time; ABI AgPath ID RT-PCR kit Primers: 1004F/1075R, TSV-P1 probe
White spot syndrome virus (WSSV)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1011F/1079R
Yellow head disease (YHD)	ABI AgPath ID RT-PCR kit; Primers: 141F/206R; YHV TaqMan
Infectious hypodermal and haematopoietic necrosis (IHHNV)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1608F/1688R
Infectious myonecrosis virus (IMNV)	ABI TaqMan One-Step RTPCR Master Mix; Primers: new412F/545R, IMNVp1
Necrotising hepatopancreatitis (NHP)	Real time: Maxima Probe/Rox qPCR Master Mix; Primers: 1300F/1366R
Nucleic acid/ Enzyme	**Detection method**
RNA extraction	MPLC Total Nucleic Acid Kit (ROCHE)
DNA extraction	MPLC Total Nucleic Acid Kit (ROCHE) & QIAGEN QIAamp DNA Mini Kit (for NHP real-time PCR)
RT-PCR enzyme	ABI AgPath ID RT-PCR kit & ABI TaqMan One-Step RTPCR Master Mix
PCR enzyme	Maxima Probe/Rox qPCR Master Mix

surveillance programs for marine food fish diseases and on-site consultation. Animal Health Laboratory Services of AVA has the capability to diagnose economically important viral diseases of penaeid shrimps (Table 1) using different molecular methods (Table 2). In addition, it has also the capability to diagnose shrimps suffering from AHPND through histopathology and isolation of its causal agent which is *V. parahaemolyticus* (VP_{AHPND}) (Table 1). The establishment of the polymerase chain reaction (PCR) assay for AHPND is currently being undertaken to further strengthen AVA's diagnostic capacity.

Disease prevention and control strategies

Singapore has an active surveillance program for WSD. Active surveillance likewise includes World Organization for Animal Health (OIE)-notifiable and emerging diseases of penaeid shrimps and fish (Agri-Food and Veterinary Authority of Singapore, 2011). The frequency of sampling for WSD is being conducted twice a year per farm establishment. The data collected from the surveillance have by far been useful in identifying disease trends and risks beneficial in the review of AVA's surveillance programs. AVA has extended the national surveillance to breeders and non-export farms since early 2009. This move has facilitated the early detection of aquatic diseases thereby allowing AVA to certify disease-free status of the farms at the national level and opened up accessibility to major markets like the European community (Agri-Food and Veterinary Authority of Singapore Annual Report 2009/2010).

The Ornamental Fish Newsletter (Agri-Food and Veterinary Authority of Singapore, 2010) cited some of the recent activities for WSD. First, WSD is notifiable to AVA under the Animals and Birds (Disease) Notification. Based on section 30 of Animals and Birds Act, offense will be imposed to a person who does not report cases of animal infected with or reasonably suspected to be infected with the disease(s). He/she shall be liable on conviction to a fine not exceeding USD 10,000 or imprisonment not exceeding 12 months or to both. Second, submission of a health certificate issued by the competent authorities of exporting countries is required in order to import ornamental aquatic animals. The requirement is applicable to ornamental species which are susceptible to seven diseases including WSD. Third, in order to increase awareness, AVA has distributed disease cards on WSD and other diseases to licensed importers and exporters. The card includes clinical signs to look out for and pictures of diseased animals. Lastly, adoption of good biosecurity measures and sanitary practices in the premises is highly recommended.

For disease control, warrant movement controls shall be imposed in cases where pathogen detection in the animal becomes positive. This step is followed by stamping out of the infected batch, together with other in-contact susceptible species. The farm has to undergo thorough cleaning and disinfection of tanks and/or ponds before being allowed to restock. Further testing will be likewise conducted to ascertain that the disease has been eradicated.

In addition, follow up actions will also be undertaken such that if detection is in the stock that is outside the quarantine area, a 2-year suspension to sell susceptible species to countries requiring the disease freedom will be strictly observed. The aforementioned premises will also undergo a minimum of twice a year sampling for that particular disease and that, suspension will only be lifted after attaining negative results for two successive years of sampling.

Way forward

In conjunction with AVA's research and development program, early detection of infectious transboundary shrimp pathogens will still be the main focus to maintain Singapore's animal disease-free status thereby facilitating the country's international trade, protecting the health of local animal populations, and more importantly, safeguarding human health. In addition, the Animal Health Laboratory Services of AVA will continually conduct its surveillance program on important shrimp pathogens especially those notifiable to the OIE.

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