Collection and Evaluation of Existing Test Methods for Acute hepatopancreatic necrosis disease (AHPND)

Background

The Regional Collaboration Framework on Aquatic Animal Health in Asia and the Pacific was developed through meetings in 2018 and 2019 and endorsed by the OIE Regional Commission for Asia, the Far East and Oceania in its 31st Conference in September 2019. At the 1st meeting of the *ad hoc* Steering Committee of the Framework (hereinafter "the Steering Committee"), the Steering Committee members discussed the activities that should be prioritized under the Framework in 2020. Noting that there are limited resources in the Region and also the importance of aquaculture biosecurity to the Region, the Steering Committee agreed with the following three themes as the regional priority topics for 2020;

- Collection and Evaluation of Existing Guidelines and Awareness Materials on Aquaculture Biosecurity for Small-scale Farms in the Asia-Pacific Region
- Collection and Evaluation of Existing Test Method for Acute hepatopancreatic necrosis disease (AHPND)
- Method of utilising OIE scientific network in emerging disease response

Acute hepatopancreatic necrosis disease (AHPND) causes high mortality in penaeid shrimp which ranges from 70% to 100% and causes a major negative impact in the shrimp culture industries. The causative agent of this disease is a specific strain of a gram negative ubiquitous bacteria, *Vibrio parahaemolyticus* (VP_{AHPND}) that contains an approximately 70-kbp plasmid, pVA1, with genes that encode homologues of the Photorhabdus insect-related (*Pir*) toxins protein A and B, *Pir*-A and *Pir*-B. VP_{AHPND} has been identified as the causative agent of AHPND, but the plasmid pVA1 also carries a cluster of genes related to conjugative transfer, which means that this plasmid is potentially able to transfer to other bacteria. Some studies have shown evidence of the horizontal transfer of pVA1-type plasmid [1-5], and there are reports that other *Vibrio* species may also cause AHPND[6]; however these other potentially AHPND-causing bacteria do not currently meet the criteria for inclusion as causative agents of AHPND. Therefore, more research is needed to demonstrate the other species of *Vibrio* carrying the *Pir*-A and *Pir*-B toxin genes could reproduce the disease AHPND

For the diagnosis of AHPND, different samples were recommended depending on the shrimp size. Diagnosis of stomach can be used for destructive sampling of broodstock, adults and juveniles. For non-destructive sampling of broodsrock, diagnosis using faecal matter was recommended. For post larvae, diagnosis of whole shrimp is favourable.

For AHPND detection methods, it can be tested using three conventional PCR detection methods: AP1, AP2 and AP3 (As an alternative to AP3, which targets the *pirA* gene, other

primer sets and protocols can also be used; these include the VpPirA-284 primer and the VpPirB-392 primer, as documented in the OIE manual, as well as the *PirA*-targeting real-time PCR assay described in Han et al (2015)[7].) Regardless of which toxin gene assay is used, there are three presentations of *V. parahaemolyticus* which are; bacteria without pVA1 plasmid, bacteria containing the pVA1 plasmid with binary toxin genes (toxin plasmid) and bacteria containing the pVA1 plasmid where the binary toxin genes have been deleted [pVA1Δpir] (deletion plasmid)[8] Previously, pVA1 plasmid is known as virulence plasmid but since it can be either virulent or non-virulent, depending on the presence of the toxin genes, it then has been proposed to use the term "toxin plasmid" for pVA1 with pir binary toxin gene and "deletion plasmid" for pVA1 without the pir binary toxin gene. AP1 and AP2 detection methods give a positive result for both toxin plasmid and deletion plasmid since both of these methods target sequences outside the toxin gene [8][9] while for AP3 PCR detection method[8][10][11] which targets directly the toxin genes sequence, it shows positive result for the toxin plasmid and negative for deletion plasmid. However, pVA1 deletion plasmid is potentially threatening because it can acquire the toxin gene easily through the process of transformation or recombination. Therefore, it is extremely important to test for both plasmids using AP1 or AP2 and the *Pir* toxin genes using AP3. The shrimp are not able to survive if the toxin genes are presence while there is possibility for the avoidance of AHPND infection in shrimp if toxin gene acquisition can be prevented. Nevertheless, strict farm biosecurity must be achieved for this prevention of acquisition toxin gene.

Objectives

1. To collect material causing AHPND and APHND-like disease from the region and evaluate existing test method for AHPND detection;
2. To collect samples or information to support the Aquatic Animal Health Standards Commission's work on re-evaluating the definition of AHPND;
   The scientific information needed are as follows:
   - Demonstration of the presence of the plasmid carrying the PirA and PirB toxin genes in other species of Vibrio,
   - Description of the re-isolation and identification of the bacteria in order to demonstrate definitively that these bacterial species could reproduce the disease AHPND.
   - Given the potentially widespread presence of the PirA and PirB genes in nature it is important to ensure that, before expanding the scope of the disease, there is definitive evidence to support any bacterial species as a pathogenic agent. Re-isolation and identification of the bacterial agent after determining the presence of the toxin genes as well as evidence to demonstrate that the bacterial species is the cause of AHPND (e.g. bioassay) is required to fulfil Koch's postulates.
3. To understand the mechanism on how the pathogenic agent *Vibrio* parahaemolyticus (Not limited to *Vibrio parahaemolyticug*) takes up the 70-kbp plasmid with genes that

encode homologues of the *Pir* toxins protein A and B, *Pir*-A and *Pir*-B, by collecting available AHPND control material from the region;

4. To develop and publish a laboratory diagnostic algorithm to address the need in the Asia-Pacific region for AHPND detection and a report to be considered by the Aquatic Animal Health Standards Commission for further improvement of existing OIE standards.

Activities

OIE Regional Representation for Asia and the Pacific (OIE RRAP) will contact OIE Delegates and Focal Points for Aquatic Animals officially through e-mail to encourage them to share the AHPND positive materials that meet the criteria* with OIE Reference Laboratory for AHPND in Chinese Taipei, International Center for the Scientific Development of Shrimp Aquaculture (CDSA). Meanwhile, evaluation and analysis of existing AHPND test method will be carried out by Dr Grace Lo in collaboration with other experts. Meeting with relevant experts will be undertaken for further discussion and assessment of the AHPND laboratory algorithm and the results of her research. All collection of results will be considered by the OIE Aquatic Animal Health Standards Commission.

*The AHPND positive materials should meet the following criteria:

1. pVA1 negative (for example, AP1 and AP2 are both negative) and toxin genes positive (for example AP3 positive).
2. pVA1 positive (for example, AP1 or AP2 positive) and toxin genes positive (for example AP3 positive) but the bacteria is not Vibrio parahaemolyticus.
3. pVA1 positive (for example, AP1 or AP2 positive) and toxin genes positive (for example AP3 positive) but the PirA/B protein is not expressed (for example, western blots are negative).

To participate in this activity, please contact Dr Grace Lo, OIE designated for AHPND and infection with white spot syndrome virus, at gracelow@mail.ncku.edu.tw and/or OIE RRAP at rr.asiapacific@oie.int.

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