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Health & Welfare
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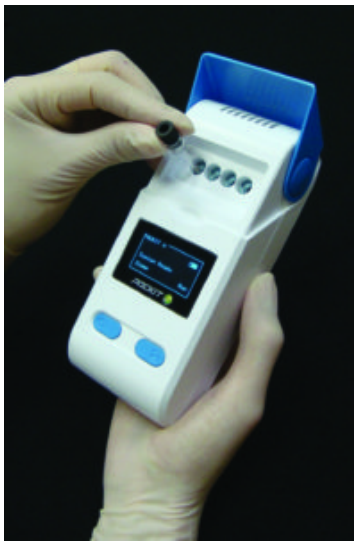
Rapid on-site diagnostic tool for AHPND management

Responsible Seafood Advocate logo
1 July 2015 Simon Chung Dr. Pei-Yu Alison Lee Li-Juan Ma

Highest mortality rates are found during the first 20 to 40 days of culture



Hand-held PCR devices are now available for rapid on-site detection of shrimp diseases.

Growing rapidly in recent decades, shrimp aquaculture is now one of the major global agriculture industries. In parallel, emerging shrimp diseases have also been on the rise, largely because shrimp are cultured at high density, exposed to environmental stress and traded globally.

Since 2009, early mortality syndrome, also termed acute hepatopancreatic necrosis disease (AHPND), has caused large-scale shrimp deaths and huge economic losses for shrimp farmers in China, Vietnam, Malaysia and Thailand. AHPND can affect *Penaeus monodon*, *P. chinensis* and *Litopenaeus vannamei*.

The highest mortality rates for shrimp with AHNPD are found during the first 20 to 40 days of culture in grow-out ponds. Typical signs of AHPND infection, which are found mainly in the digestive tracts of shrimp, include an empty gastrointestinal tract and abnormal shrunken, whitish hepatopancreas, which result in weakness and death in shrimp. AHPND infection is defined by histological changes in the hepatopancreatic system, particularly sloughing of hepatopancreas tubule epithelial cells.

In 2013, the causative agent of AHPND/EMS (AHPND *V.ph*) was identified as a unique pathogenic strain of *Vibrio parahaemolyticus* containing a virulence-associated plasmid. *V. parahaemolyticus* has been in marine environments worldwide.

Molecular identification

Recently, *toxin 1*, a homolog of an insecticidal *toxin*, has been shown to play a critical role in hepatopancreas damage caused by AHPND bacteria in diseased shrimp. *Toxin 1* consists of two genes, pirA and pirB, and pirB alone could cause the typical histological changes associated with AHPND in shrimp hepatopancreas.

Virulence factor genes, including *toxin 1*, have been found within "pathogenic islands" flanked by inverted repeats of transposase genes in the so-called AHPND-associated plasmid. Therefore, both *toxin 1* (pirA and pirB) and the AHPND-associated plasmid are potential markers to allow identification of virulent AHPND bacteria. The AHPND-associated plasmid could be identified by the presence of certain unique markers.

Various molecular methods targeting *toxin 1* and/or the AHPND-associated plasmid are available to help APHND diagnosis. However, different *Vibrio* species readily exchange genetic materials among themselves through genetic transfer mechanisms, including homologous recombination, transposition, conjugation or transformation. Transposases can move genes horizontally from one location to another location.

So far, non-virulent *V. parahaemolyticus* strains containing truncated AHPND-associated plasmids with *toxin 1* deletion have been found in the environment. Therefore, detection of *toxin 1* alone is not recommended, since *toxin 1* is located in an unstable region.

Although no evidence has been reported yet, it is suspected that the *toxin 1* gene region could be transferred to another bacteria. And the new *toxin 1*-harboring bacteria might not be virulent, because AHPND pathogenesis may need other virulence factors.

On the other hand, it has been reported that 98 percent of the samples tested AHPND-associated plasmid positive by polymerase chain reaction were found to show AHPND symptoms. Therefore, detection of the plasmid alone could help detect a potential AHPND threat. Thus, to diagnose AHPND, detection of both the plasmid and *toxin 1* gene is recommended.

When simultaneous performance of the two assays is not possible, due to cost concerns, for example, it is recommended to perform screening for the plasmid first to identify a potential threat. In case of plasmid-positive results, the *toxin 1* assay could be used to provide further evidence of AHPND.

A good AHPND detection method also should not cross-react with host DNA, any irrelevant plasmids and other microorganisms. Because not much bioinformatics information is available for *toxin 1* homologs, probe-based molecular methods should offer better specificity than conventional methods to detect targets of this nature.

On-site molecular detection tools

Without vaccines and effective treatments for shrimp diseases, the shrimp industry relies greatly on biosecurity measures to prevent the introduction of pathogens or risk factors to facilities to reduce the spread of pathogens throughout the culture environment.

Polymerase chain reaction (PCR) assays, with their high sensitivity and specificity, have been employed commonly at various large-scale aquaculture facilities for pathogen surveillance or disease diagnosis. This approach has proven to help detect pathogens at early stages to facilitate timely implementation of measures to control the spread of major shrimp disease agents such as white spot syndrome virus, improving overall shrimp production in the long run.

However, due to the challenging requirements for sophisticated equipment and highly trained personnel to perform PCR, small-scale farms in general have been reluctant to include PCR assays in their biosecurity measures. Therefore, user-friendly, field-deployable detection methods are now available to monitor and improve biosecurity at small-scale farms.

Optimal on-site detection systems should be rapid, inexpensive, sensitive and easy to maintain and perform for anyone with minimal training. In addition, the reagents should be provided in a format that allows easy shipping and storage.

Current on-site method

A molecular diagnostic assay based on insulated isothermal polymerase chain reaction (iiPCR) testing, working on a specific field-deployable device, is currently available for on-site detection of AHPND. The reliability of the iiPCR system has been demonstrated by a similar system certified by the World Organisation for Animal Health (OIE) as validated for detecting white spot syndrome virus in shrimp in 2013.

The portable device can amplify target sequence, detect fluorescent signals and display simple readouts within one hour. Two iiPCR assays, targeting either the most stable region of the AHPND plasmid or the virulence-related *toxin 1*, have been developed to monitor the markers in shrimp, water, live feed or other sources to help evaluate risks for AHPND.

The assays detect their targets sensitively and specifically. For example, the sensitivity of both iiPCR assays were comparable to that of real-time PCR assays, based on results of an endpoint dilution study using serial dilutions of a virulent AHPND V.ph strain (Table 1).

Chung, DNA dilutions, Table 1

DNA Dilutions	toxin 1: Real-Time PCR	toxin 1: iiPCR Assay	AHPND Plasmid Real-Time PCR	AHPND Plasmid iiPCR Assay
-1	2/2	2/2	2/2	2/2
-2	2/2	2/2	2/2	2/2
-3	3/3	2/2	3/3	2/2
-4	0/3	0/3	0/3	2/3
-5	0/3	0/3	0/3	0/2

Table 1. Sensitivity performance of iiPCR assay for the virulent AHPND V.ph.

For specificity evaluation, cultures of 18 of the AHPND strains were tested. The *toxin 1* iiPCR reacted positively with all eight *toxin 1*-positive strains and negatively with all 10 *toxin 1*-negative strains identified by a reference *toxin-1* PCR assay from National Cheng Kung University in Tainan, Taiwan, indicating this reagent has excellent specificity in detecting the virulence marker of the AHPND V.ph.

Perspectives

Good diagnostic systems are mostly built on well-established technology and research results. Reagents should be manufactured under controlled environments with quality controls following international standard procedures and pass stringent validation procedures.

Application of these systems in pathogen detection can often be performed only on specific samples at specific time points. However, shrimp cultures generally involve huge shrimp populations associated with dynamic conditions over long periods of time. Therefore, to get the most of these tools, the users should also pay attention to sampling site, size and timing. Furthermore, for correct diagnosis of a disease, it is best to analyze the samples using multiple test methods based on different technologies, such as microbiology, histopathology and qualitative/quantitative PCR.

On the whole, bacteria such as the AHPND *V.ph* are hard to eliminate and could have long-term impacts on shrimp farms once they are introduced into the facilities. Screening and removal of potential AHPND threats from shrimp, water, live feeds or other sources is critical in biosecurity. On-site diagnosis should make the implementation of this important measure possible at shrimp culture facilities of different scales at any location.

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