

FINAL REPORT

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HOST ORGANISATION: CSIRO AAHL

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ACTIVITY UNDERTAKEN

I attended the 2017 Shrimp Pathology short course held in Tucson, Arizona (17th-22nd July). The short course discussed the major shrimp pathogens through a series of lectures and practical demonstrations. The course covered histopathology, molecular techniques and an AHPND bioassay.

OUTCOMES ACHIEVED TO DATE

Through the broad range of lectures and practical demonstrations during the short course, I have gained the knowledge and experience in identifying the major pathogens of farmed shrimp. I have further advanced my knowledge of the major shrimp pathogens, and the disease pathology they present in the animals. In addition to understanding the key histopathological features, I have gained a strong understanding of how the OIE reference laboratory processes samples for disease detection and diagnosis using molecular techniques.

Furthermore, through the undertaking of a bioassay, I am aware of the different ways to perform shrimp bioassays to further understand the pathogenicity of important shrimp pathogens. With the new knowledge and skills gained, I will apply my experience to current activities in the laboratory to improve molecular assays where possible through comparison of difference extraction techniques and molecular tests to ensure diagnostic performance. In addition, alternative bioassay methods will be discussed and analysed to ensure optimal performance in each pathogenicity trial is achieved. I believe the outcome of the training course will further strengthen both our molecular and bioassay activities to a level that is equal to or greater than other OIE reference laboratories.

Acknowledgments

I would like to thank the FRDC Animal Health and Biosecurity Subprogram/DAWR for funding the training grant; CSIRO for allowing me to undertake the training at the OIE world reference laboratory; and the University of Arizona for holding the 2017 shrimp pathology short course.

Background

I completed my PhD in molecular biology in 2009 from the University of Melbourne. Since joining AFDL in 2013, I have been undertaking molecular testing for aquatic animal pathogen detection and identification and have also been involved in molecular assay development. With respect to pathogens of farmed shrimp I have been part of the team undertaking testing for both AHPND and WSSV. Knowledge and skills gained from this training course will be directly applied to our current activities including, (1) Knowledge gained from molecular techniques will be used for benchmarking in AFDL to compare methods, in particular, comparing their U. Arizona WSSV PCR extraction and PCR testing systems to our current existing protocols and (2) The method used for the bioassays will be compared with the protocols used in our secure animal facility with not only for AHPND but also for other prawn diseases to ensure successful challenge and onset of clinical disease for pathogenicity studies and generation of material for validation of assays according to OIE guidelines.

Need

Shrimp diseases have significantly impacted shrimp (prawn) production throughout Asia, while Australia has remained relatively untouched by the economic impacts of these diseases. However, in 2016, farms in two regions of Queensland were affected by a bacterial disease similar to acute hepatopancreatic necrosis disease (AHPND) resulting mass mortalities. Then, in November 2016, an outbreak of white spot syndrome virus (WSSV) was confirmed in farms located along the Logan River in SE Queensland. The disease spread to all eight prawn farms along the Logan River resulting in the destruction of all stock and decontamination of the farms.

The aquaculture pathology laboratory at the University of Arizona is an OIE Reference Laboratory for numerous prawn diseases. AFDL is currently an OIE Reference Laboratory for infection with yellow head virus genotype 1 (YHV1), with ongoing discussions on becoming an OIE Reference Laboratories for AHPND and WSSV. It is therefore important to ensure that we are at the forefront of prawn disease diagnosis.

With the recent outbreak of AHPND-like disease and WSD, more focus will be placed on these diseases in the next couple of years through (1) ensuring the best diagnostic tests are on-hand, and (2) experimental animal trials (bioassays) to determine the susceptibility of Australian prawns to the exotic strains of AHPND and WSSV.

In addition to WSSV and AHPND, there are a number of other prawn disease diagnostic tests in the OIE Manual that do not perform as well as an OIE test should. These assays will be discussed during the training course with the hope that on return to AFDL I will be able to improve their performance in the laboratory.

Objectives

(1) Understand the workflow, process and methods the OIE reference laboratory undertakes for disease diagnosis. In particular, how they test for WSSV and issues relating to PCR inhibitors. This will allow for comparison testing to be undertaken to ensure our methodology is equivalent.

(2) Apply the bioassay experiment methodology used at the aquaculture pathology laboratory to undertake a comparison study to ensure our current bioassay method is equivalent in terms of producing disease in susceptible animals.

(3) Gain knowledge and discuss issues about the diagnostic tests used for other prawn pathogens for which they are the OIE reference experts. This would allow us to improve poorly performing tests for which there is no other designated assay described or determine whether new assays need to be developed.

Methods

The 2017 Shrimp pathology short course consisted of both theory (lectures) and hands-on (lab practical) activities. A broad range of shrimp disease was covered and included in the following structure:

Lectures: The following topics were covered:

1. Short-course introduction, purpose, scope and schedule.
2. Major shrimp diseases listed by World Animal Health Organization (OIE)
3. The baculovirus diseases: white spot syndrome virus (WSSV), monodon-type baculovirus (MBV), baculovirus penaei (BP)
4. The parvovirus diseases: infectious hypodermal and hematopoietic necrosis virus (IHHNV), hepatopancreatic virus (HPV)
5. RNA viruses infecting penaeid shrimp: Taura syndrome virus (TSV), yellow head virus (YHV), infectious myonecrosis (IMNV), *Penaeus* nodavirus (PvNV), white tail disease (WTD)
6. Bacterial diseases: acute hepatopancreatic necrosis disease (AHPND; caused by a unique strain of *Vibrio parahaemolyticus*), necrotizing hepatopancreatitis (NHP-B), *Vibrio harveyi*.
7. Microsporidian diseases: *Enterocytozoon hepatopenaei* (EHP), cotton shrimp disease
8. Methods of disease prevention and/or treatment.
9. Development of biosecurity and quarantine protocols

Labs and Demonstrations: Topics were covered in the labs included

1. Sample preparations for histology and PCR.
2. PCR/RT-PCR for diagnosis of WSSV, AHPND, EHP, TSV
3. qPCR/qRT-PCR for diagnosis of WSSV, AHPND, TSV
4. Laboratory bioassay: AHPND
5. Review of histopathology of viral and bacterial diseases

Results

(1) Understand the workflow, process and methods the OIE reference laboratory undertakes for disease diagnosis. In particular, how they test for WSSV and issues relating to PCR inhibitors. This will allow for comparison testing to be undertaken to ensure our methodology is equivalent.

Objective 1 was achieved through the practical demonstrations and additional discussions with the UOA staff.

(2) Apply the bioassay experiment methodology used at the aquaculture pathology laboratory to undertake a comparison study to ensure our current bioassay method is equivalent in terms of producing disease in susceptible animals.

Objective 2 bioassay details used in the training course will be tested alongside our existing methods to compare pathogenicity of AHPND using alternative infection methods in the next bioassay experiment. Results from this comparison trial will shape future bioassay experiment protocols.

(3) Gain knowledge and discuss issues about the diagnostic tests used for other prawn pathogens for which they are the OIE reference experts. This would allow us to improve poorly performing tests for which there is no other designated assay described or determine whether new assays need to be developed.

Objective 3 was achieved from understanding and gaining knowledge into the methods used to detect various shrimp pathogens using molecular techniques. It is also anticipated that Objective 3 will further be achieved through a collaboration with the University of Arizona to work together to improve shrimp diagnostic tests.

Discussion

By participating in the intensive shrimp pathology short course, I have an increased awareness and familiarity of the major pathogens that affect the shrimp farming industry. I am competent in collecting samples and preparing them for diagnostic analysis. I am able to identify the major pathogens using histopathological techniques. I have also further enhanced my molecular skills through understanding alternative methods and assays that are used to identify the presence of shrimp disease agents using both conventional and real-time PCR. I have also gained experience in undertaking bioassays using an alternative method which may enhance the effectiveness of future bioassays undertaken within CSIRO AAHL Fish Diseases Laboratory. Of particular benefit, beyond the skills and knowledge gained through attending the short course, is the further development of our network and the potential for future collaboration between the OIE Reference Laboratories to improve shrimp disease diagnostics and knowledge.

Benefits and Adoption

The following outcomes will be achieved following the 2017 Shrimp Pathology Course:

(1) An understanding of the workflow, process and methods the OIE Reference Laboratory undertakes for disease diagnosis was achieved, in particular, how UoA tests for WSSV and issues relating to PCR inhibitors will allow for comparison testing of UoA and AFDL procedures to be undertaken to ensure AFDL's methodology is at least equivalent with respect to diagnostic sensitivity and specificity.

(2) AFDL will have the ability to apply the bioassay experiment methodology used at the aquaculture pathology laboratory to undertake a comparison study to ensure our current bioassay method is at least equivalent in terms of producing disease (with typical mortality rates, histopathology and time course) in susceptible animals.

(3) By gaining increased knowledge of specific aspects of the diagnostic tests and through face-to-face discussion of issues concerning the diagnostic tests used for other prawn pathogens for which UoA is the OIE Reference Laboratory allows us to improve any poorly performing tests for which there is no other designated assay described or, alternatively, allows us to determine whether new assays need to be developed.

By ensuring that both our diagnostic testing standards and experimental procedures are to the same/or higher standard to that of other OIE Reference Laboratories, that our testing performs as expected and consistently, both industry (e.g. Australian Prawn Farmers Association) and state and federal governments will remain confident in the work (both research and diagnostic testing) AFDL undertakes. Providing reassurance that the AAHL Fish Diseases Laboratory is capable of improving and responding to future disease outbreaks.

Further Development

In addition for the new skills and knowledge gained through this training, it is anticipated that both CSIRO AAHL and the University of Arizona (Aquaculture Pathology Laboratory) will collaborate on the development of new and improved shrimp assays.

References

NA

Intellectual property

NA

Appendices

NA