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Testing uncooked retail prawn commodities imported into Australia for the presence of Taura Syndrome Virus (TSV) and Yellowhead Virus 1 (YHV1)

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FRDC Project 2017-088

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In submitting this report, the researcher has agreed to FRDC publishing this material in its edited form.

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Acronyms

ALOP	Appropriate Level of Protection
APFA	Australian Prawn Farmers Association
DAWR	Australian Government, Department of Agriculture and Water Resources
IRA	Import Risk Analysis
IGB	Inspector General of Biosecurity
IIGB	Interim Inspector General of Biosecurity
OCVO	Office of the Chief Veterinary Officer
OIE	Office International des Epizooties World Organisation for Animal Health
qPCR	Quantitative Polymerase Chain Reaction (real-time PCR)
WTOSPS	World Trade Organisation Sanitary and Phytosanitary Agreement
TSV	Taura Syndrome Virus
WSSV	White Spot Syndrome Virus
WSD	White Spot Disease
WTO	World Trade Organisation
YHV	Yellowhead Virus

Glossary

Fenneropenaeus merguiensis	Banana prawn
Haliporoides sibogae	Royal red prawn
Litopenaeus vannamei	Pacific white shrimp
Melicertus latisulcatus	King prawn
Metapenaueus benettae	Greentail prawn
Peneaus esculentus	Brown tiger prawn
Peneaus monodon	Black tiger prawn
Scylla serrata	Mud crab

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Executive Summary

What the report is about

As part of the response to the outbreak of White Spot Disease (WSD) in late November 2016, uncooked prawns were purchased by Dr Matt Landos (Future Fisheries Veterinary Service Pty Ltd) from various retail outlets in northern NSW and south east Queensland and these were tested by qPCR for White Spot Syndrome Virus (WSSV) under FRDC project 2016/066. This project utilised residual sample material which had been held at -80oC at Sydney University since collection during December 2016 and January 2017. All remaining samples which were identifiable as imported were tested by PCR for two further exotic crustacean viruses: Yellowhead Virus-1 (YHV-1) and Taura Syndrome Virus (TSV).

Background

Until 2016, the Australian prawn farming industry had been fortunate to remain free from many of the serious viral diseases which have decimated prawn farms internationally. In November 2016 an outbreak of WSSV occurred on a prawn farm on the Logan River, SE Queensland and quickly spread resulting in State Government controlled destruction of all prawns in all farms in the region as part of an eradication emergency disease response. The Fisheries Research and Development Corporation (FRDC) supported a project (2016/066) exploring the risks associated with imported raw prawn commodities as an entry pathway for WSSV into Australia. Testing of the retail commodities for WSSV conducted during this project revealed a high proportion (~86%) were positive by qPCR for WSSV. The WSSV outbreak and other concerns around non-compliant importation practices led to a cessation of the imported uncooked trade temporarily in January 2017.

As a consequence of the State and Commonwealth investigation of the WSSV outbreak on the Logan River in SE Queensland prawn farms from late November 2016, it became apparent that a significant number of recreational anglers were purchasing imported uncooked prawns from supermarkets and other seafood outlets and using these as bait although these products were labelled as “For human consumption only - not to be used as bait”. Recreational anglers in the immediate vicinity of the outbreak were observed to be using these prawns as bait, against the label recommendations. Some of these confiscated packets of “human consumption” grade prawns subsequently tested positive for WSSV.

Pathways for movement of prawn diseases have been described through the movement of commodities destined for human consumption, such as uncooked prawns. Diversion of such commodities away from human consumption, through use as bait or berley by anglers has the potential to generate a release pathway that could see these diseases enter Australian wild stocks. It also poses an entry risk to prawn farms via their intake of water, or through use of wild broodstock who may develop sub-clinical infections that are passed to PL. Recent surveys have suggested that recreational angler use of supermarket origin uncooked imported prawns for bait has increased.

The WSSV outbreak in Australia has raised concerns that the frozen commodity imported uncooked prawns may also be harbouring other potentially infectious prawn viruses such as YHV-1 and TSV. If present in imported prawn commodities, these viruses, like WSSV, pose a significant risk to the Australian prawn farming industry as the lack of compliance to the import requirements combined with a release pathway via use of imported prawns for bait and burley has been demonstrated.

Aims/objectives

Determine the PCR test status of a range of imported uncooked prawn commodities with respect to the presence of YHV-1 and TSV.

Utilise the knowledge gained to inform the response of the Australian Prawn Farmers Association (APFA) into the review of the crustacean Import Risk Assessment in 2018. Data from this testing will also assist farmers in relation to making risk based decisions around major capital expenditure on farms to increase biosecurity and surveillance.

Knowledge of the risks associated with uncooked prawn importations will assist the industry in contributing to the review of the Import Risk Assessment of uncooked crustacean which is currently underway.

Methodology

DNA extracts and source prawn tissues from 104 imported prawn commodities are being held in -80oC freezers. Each commodity had up to 2 pools per commodity tested for TSV and YHV-1 by PCR. Methods used aligned with those described by the OIE Diagnostic Manual. There were 206 pooled (up to 5 animals per pool) prawn product samples selected for testing that were predominantly derived from muscle tissue. Testing was completed between August and October 2017 at the Sydney University, Infectious Diseases Laboratory, Faculty of Veterinary Science, Animal and Veterinary Public Health, Camden, NSW.

Results/key findings

YHV-1: All samples tested negative.

TSV: There were no confirmed positive results. There were 15 samples that produced a high Ct values (>35) in one or more replicates of the first test. Each of these samples tested negative when an additional nucleic acid extract was prepared from the tissue homogenates stored at -80°C. This is consistent with a very low quantity of the virus being present in the sample. Thus, the results for these 15 samples are reported as inconclusive.

Re-testing of the 15 inconclusive samples was undertaken at NSW Primary Industry, Elizabeth Macarthur Agriculture Institute. All returned negative results for TSV.

Implications for relevant stakeholders

The samples tested appear to pose a negligible risk with respect to the transmission of TSV and YHV-1 into Australia. It should be recognised that this is a small snapshot of commodities which are being imported, and may not be representative of the disease status of all imported material.

Caution is urged when interpreting uncooked imported prawns as a low risk commodity based on this study. The authors note that there are other prawn diseases which we were not able to test for, but may pose a risk in imported frozen prawn commodities.

Recommendations

Given the observed non-compliance of imported frozen commodity prawns to Australia's importation standards in 2016 to early 2017, it remains prudent for the APFA to continue to undertake periodic surveillance for important prawn pathogens in uncooked imported prawns.

The implementation of an import requirement to cook all imported prawns would result in a substantial risk reduction for prawn disease transfer into Australia, given the observed non-compliance of recreational fishers using imported prawns as bait and berley in waters in close proximity to water intakes of prawn farms.

Keywords

Yellowhead Virus- 1; YHV-1; Taura Syndrome Virus; TSV; PCR; imported frozen commodity prawn; *Penaeus monodon*; Black Tiger Prawn

Introduction

The Australian prawn farming industry has been fortunate to remain free from many of the serious viral diseases that have decimated prawn farms internationally. It had been free from WSSV until the November 2016 when virus was discovered causing disease in prawn farm on the Logan River in southeast Queensland.

In response to the outbreak, FRDC and the APFA supported a project exploring the risks associated with imported uncooked prawn commodities (FRDC Project Number 2016/066) as an entry pathway for WSSV into Australia. In January 2017, this outbreak and other concerns around illegal importation practices led to a temporary cessation of the trade of imported uncooked prawns. In this project testing of imported uncooked prawn commodities collected from retail outlets in Northern NSW and SE Queensland that were tested by qPCR at Sydney University's NATA accredited laboratory identified that a high proportion of uncooked crustacean seafood commodities contained WSSV DNA. The DNA extracts from these commodities have since been stored in -80°C freezers at Sydney University. The Commonwealth Government have now announced a recommencement of this trade in uncooked prawns with some additional testing requirements required from early July 2017. The prawn farming industry is concerned about the potential carriage of other pathogenic viruses such as Taura Syndrome Virus (TSV) and Yellow Head Virus 1 (YHV-1) for which PCR tests are available for screening the retail sourced material.

Pathways for movement of disease have been described through the movement of commodities destined for human consumption, such as uncooked prawns. Diversion of such commodities through use as bait or berley by anglers has the potential to generate a release pathway that could see these diseases enter prawn farms via their intake of water, or through use of wild broodstock who may develop sub-clinical infections. Recent surveys have suggested that recreational angler use of supermarket origin uncooked prawns for bait has increased, even though such commodities are labelled that they are intended for human consumption only, and are specifically not to be used for bait.

Broader knowledge of the risks associated with uncooked prawn importations will assist the industry in contributing to the review of the Import Risk Assessment of uncooked crustacea which is currently underway. Data from this testing will also assist farmers in relation to making risk based decisions around major capital expenditure on farms to increase biosecurity and surveillance in response to the new paradigm initiated by WSSV.

Objective

Generate data on the PCR test status of imported uncooked prawns at retail in relation to Taura Syndrome Virus and Yellow Head Virus 1, to inform APFA submissions to the review of the Commonwealth Import Risk Assessment of uncooked crustacea.

Method

Samples

The study was conducted using stored nucleic acid extracts and tissue homogenates produced from samples collected in December 2016 - January 2017 during FRDC Project 2016-066, 'Assessing compliance and efficacy of import conditions for uncooked prawn in relation to White Spot Syndrome Virus (WSSV) through testing retail commodities and comparison of stringency of import measures with other imported commodities into Australia'. The samples of imported prawns selected for testing were a subset of those obtained in this study. Two pools of 5 prawns for selected commodities were tested for Yellow Head Virus Genotype 1 (YHV-1) and Taura syndrome Virus (TSV) (Table 1).

Selected samples were prepared as pools of 5 prawns with careful attention to the work flow to ensure there was no cross-contamination between commodities and to maximise preservation and subsampling of viral nucleic acids. Briefly, equal amounts of tissue from each prawn in the pool up to a total of 0.5g were disrupted in 1 ml of RLT buffer (Qiagen) and 5 µl Dx reagent (Qiagen) by bead beating using a Tissue Lyser machine (Qiagen). The supernatant was removed after centrifugation at 12 000 g for 2 min and a volume representing 50 mg of tissue was used as the staring sample for nucleic acid purification. The All-For-One Vet Biosprint Nucleic Acid Extraction Kit (Qiagen) was used with a Biosprint magnetic particle workstation (Qiagen). A small number of samples required additional nucleic acids that were obtained by repeating the procedure using additional aliquots of tissue homogenate supernatant that were archived at -80°C.

Conventional PCR for Yellow Head Virus Genotype 1 (YHV-1)

Samples were tested according to the present recommendation in the OIE Manual of Diagnostic Tests for Aquatic Animal Disease (OIE, 2016). Assay 1 is a conventional RT-PCR specific for YHV Genotype 1 that was conducted according to the methods described by Mohr et al., (2015). Samples were tested in 25 µl reactions prepared with the Superscript/Platinum Taq one-step RT-PCR kit (Invitrogen). Reactions contained the primer 10F (5'-CCGCTAATTTCAAAAACTACG-3') and reverse primer 144R (5'-AAGGTGTTATGTCGAGGAAGT-3') at concentration of 180 nm and 2 µl nucleic acid template. Thermocycling was conducted with a conventional PCR machine (Corbett) according to the following protocol: 50°C for 30 min (reverse transcription); 94°C for 2 min; then 40 cycles of 94°C for 30s, 58°C for 45s, 68°C for 45s and a final elongation at 68°C for 7 min.

PCR products (10 µl) were loaded into a 2% agarose gel stained with RedSafe (iNtRON Biotechnology) and subject electrophoresis. Amplification products were visualised with a GelDoc transilluminator (Biorad) and examined for the presence of the 135 bp target amplicon ORF1b region of the viral genome.

Positive control RNA was prepared with the RiboMax Largescale RNA synthesis kit (Promega) using a DNA Ultramer oligonucleotide template that contained 168 bases including all of the YHV-1 target sequence and the T7 promoter used for RNA synthesis (Integrated DNA Technologies). Negative control samples included nucleic extractions performed on tissue free samples prepared during tissue processing and no template control.

Real-time PCR for Taura Syndrome Virus (TSV)

Samples were tested according to a present recommendation in the OIE Manual of Diagnostic Tests for Aquatic Animal Disease (OIE, 2017). The real-time reverse transcriptase (RT-qPCR) assay with Taqman detection chemistry was described by Tang et al., (2004). Duplicate 25 µl reactions were prepared with the AgPath-ID One-step RT-qPCR Kit (Life Technologies). The forward primer (TSV1004F: 5'-TTGGGCACCAAACGACATT-3') and reverse primer (TSV1075R: 5'-GGGAGCTTAAACTGGACACACTGT-3') were each used at a final reaction concentration of 300 nm. The probe (TSV-P1: 5'- CAGCACTGACGCACAATATTCGAGCATC -3') was labelled with a 5' FAM reporter 3' BHQ-1 quencher and used at 100 nm. The reactions were prepared with 5 µl of nucleic acid template.

Thermocycling was conducted on an Mx3000P qPCR system with the following conditions: 45°C for 10 minutes (reverse transcription); 95°C for 10 minutes (hot start activation); then 40 cycles of denaturation at 95°C for 15 s and annealing /extension/fluorescent data acquisition at 60°C for 60 s.

The FAM fluorescence signal was normalized to the ROX passive reference dye and corrected to a baseline according to the Stratagene software. A threshold fluorescence was calculated according to the amplification of the positive control by Mx3000 software. A negative result was assigned to samples in which the FAM signal did not exceed the threshold. When the FAM signal exceeded the threshold in either replicate a Ct value was assigned after visual examination of the raw fluorescence plots. A positive result was assigned to samples with a Ct value less than 40 in both replicates and when the result was repeated on a second nucleic acid template. If only one replicate was positive and/or the positive result was not repeated for an additional nucleic acid preparation, the result was determined to be inconclusive. Results were reported dichotomously as positive or negative. The average Ct value was included as this can provide some indication of the relative quantity of TSV RNA in a sample even though quantification was not performed.

Positive control RNA was prepared with the RiboMax Largescale RNA synthesis kit (Promega) using a DNA Ultramer oligonucleotide template that contained 168 bases including all of the TSV target sequence and the T7 promoter used for RNA synthesis (Integrated DNA Technologies). Negative control samples included nucleic extractions performed on tissue free samples prepared during tissue processing and no template control.

Results

There were 206 pooled seafood product samples selected for testing that were predominantly derived from muscle tissue (See Table 1). It was noted that this tissue type is less than ideal for detection of the YHV-1 and TSV target viruses, but was necessary to assess the risk of this specific import commodity pathway. The tissue type of the seafood product samples was muscle, muscle with shell and pleopod with gill for 154, 42 and 10 pooled product samples, respectively.

Previously reported results for the Vet Max Xeno-VIC internal positive control assay (Life Technologies) indicate that inhibitors of PCR were not present in the nucleic acid samples examined in the present study. All additional extractions conducted for this study included the internal positive control and produced expected results.

Yellowhead Virus 1 (YHV-1) Testing Results

All 206 pooled seafood product samples tested negative to YHV-1 (See Table 1).

The negative and positive control samples provided expected results i.e. no amplification products and a 135 base pair DNA band, respectively. There were 2 samples that produced amplicons that were <135 base pairs on high resolution gel electrophoresis.

Taura Syndrome Virus (TSV)

There were no confirmed positive results in the 206 pooled seafood product samples tested (See Table 1). There were 15 samples that produced a high Ct values (>35) in one or more replicates of the first test. Each of these samples tested negative when an additional nucleic acid extract was prepared from the tissue homogenates stored at -80°C. This is consistent with a very low quantity of the virus being present in the sample. Notwithstanding, further validation data is required for an accurate estimate of the diagnostic sensitivity and specificity. Thus, the results for these 15 samples are reported as inconclusive.

The country of origin of inconclusive samples were from China, Malaysia, Vietnam and not available (but described at point of sale as “imported”) for 6, 4, 2 and 3 pooled product samples, respectively.

The inconclusive samples were from *Litopenaeus vannamei* and not available for 11 and 4 unknown pooled product samples, respectively.

Table 1: Samples tested for YHV-1 and TSV and results of PCR tests.

Sample							Result			
Commodity	**SVC #.**	**Pool**	**Sample type**	**Species**	**Country**		**YHV-1**	**TSV**	**Ct (Av)**	**TSV Retest**
93. ML8	17/008	2	muscle	*Litopenaeus vannamei*	Malaysia		Negative	Inconclusive	38.62	Negative
		3	muscle	*Litopenaeus vannamei*	Malaysia		Negative	Negative	No Ct	Not tested
121. ML36	17/008	9	muscle	*n/a*	Vietnam		Negative	Negative	No Ct	Not tested
		10	muscle	*n/a*	Vietnam		Negative	Negative	No Ct	Not tested
136. ML51	17/008	14	muscle w shell	*Litopenaeus vannamei*	China		Negative	Negative	No Ct	Not tested
		15	muscle w shell	*Litopenaeus vannamei*	China		Negative	Negative	No Ct	Not tested
118. ML33	17/008	18	muscle	*Litopenaeus vannamei*	China		Negative	Negative	No Ct	Not tested
		19	muscle	*Litopenaeus vannamei*	China		Negative	Inconclusive	38.89	Negative
117. ML32	17/008	25	muscle	*Litopenaeus vannamei*	China		Negative	Inconclusive	37.71	Negative
		26	muscle	*Litopenaeus vannamei*	China		Negative	Negative	No Ct	Not tested
127. ML42	17/008	33	muscle	*Litopenaeus vannamei*	Malaysia		Negative	Negative	No Ct	Not tested
		34	muscle	*Litopenaeus vannamei*	Malaysia		Negative	Negative	No Ct	Not tested
132. ML47	17/008	37	muscle	*Litopenaeus vannamei ?*	Vietnam		Negative	Negative	No Ct	Not tested
		38	muscle	*Litopenaeus vannamei ?*	Vietnam		Negative	Negative	No Ct	Not tested
135. ML50	17/008	42	muscle	*Litopenaeus vannamei*	China		Negative	Negative	No Ct	Not tested

		43	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
88. ML3	17/008	48	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		49	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
134. ML49	17/008	56	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		57	muscle	*Litopenaeus vannamei*	China		Negative		Inconclusive	39.47	Negative
104. ML19	17/008	60	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Inconclusive	37.01	Negative
104. ML19	17/008	61	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
119. ML34	17/008	69	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		71	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
91. ML6	17/008	75	muscle w shell	*Litopenaeus vannamei*	China		Negative		Inconclusive	36.04	Negative
		77	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
108. ML23	17/008	78	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
		79	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
105. ML20	17/008	84	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		85	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
131. ML46	17/008	90	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		91	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
103. ML18	17/008	154	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		155	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested

111. ML26	17/008	160	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		161	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
130. ML45	17/008	168	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		169	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
109. ML24	17/008	172	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
		173	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
90. ML5	17/008	178	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
		179	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
112. ML27	17/008	182	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		183	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
106. ML21	17/008	187	muscle	*Litopenaeus vannamei ?*	Vietnam		Negative		Negative	No Ct	Not tested
		188	muscle	*Litopenaeus vannamei ?*	Vietnam		Negative		Negative	No Ct	Not tested
113. ML28	17/008	194	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		195	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
120. ML35	17/008	199	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
		200	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Inconclusive	39.64*	Negative
95. ML10	17/008	205	muscle	*n/a*	Malaysia		Negative		Negative	No Ct	Not tested
		206	muscle	*n/a*	Malaysia		Negative		Negative	No Ct	Not tested
107. ML22	17/008	212	muscle	*Litopenaeus vannamei ?*	China		Negative		Negative	No Ct	Not tested

		213	muscle	*Litopenaeus vannamei ?*	China		Negative		Negative	No Ct	Not tested
133. ML48	17/008	217	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		218	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
110. ML25	17/008	222	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
		223	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
89. ML4	17/008	124	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		126	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
137. ML52	17/008	130	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
		131	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
94. ML9	17/008	136	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		137	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
76. NKe50	17/010	258	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		259	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
77. NKe51	17/010	264	muscle	*Litopenaeus vannamei*	Thailand		Negative		Negative	No Ct	Not tested
		265	muscle	*Litopenaeus vannamei*	Thailand		Negative		Negative	No Ct	Not tested
82. NKe56	17/010	296	muscle	*Litopenaeus vannamei*	China		Negative		Inconclusive	39.96*	Negative
		297	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
67. NKe41	17/010	308	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		309	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested

80. NKe54	17/010	314	muscle	*n/a*	Imported		Negative		Negative	No Ct	Not tested
		316	muscle	*n/a*	Imported		Negative		Negative	No Ct	Not tested
29. NKe3	17/010	348	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		349	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
51. NKe25	17/010	362	muscle	*n/a*	Vietnam		Negative		Inconclusive	39*	Negative
		363	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
62. NKe36	17/010	368	muscle	*Litopenaeus vannamei*	n/a		Negative		Negative	No Ct	Not tested
		369	muscle	*Litopenaeus vannamei*	n/a		Negative		Inconclusive	37.71*	Negative
61. NKe35	17/010	379	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		380	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
57. NKe31	17/010	385	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		386	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
68. NKe42	17/010	391	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
		392	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
59. NKe33	17/010	448	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
		449	muscle	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
78. NKe52	17/010	457	muscle	*n/a*	Imported		Negative		Negative	No Ct	Not tested
		458	muscle	*n/a*	Imported		Negative		Negative	No Ct	Not tested
34. NKe8	17/010	529	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested

		530	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
28. NKe2	17/010	547	muscle	*Tiger prawn*	Imported		Negative		Negative	No Ct	Not tested
		548	muscle	*Tiger prawn*	Imported		Negative		Negative	No Ct	Not tested
55. NKe29	17/018	565	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		567	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
53. NKe27	17/018	569	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
		570	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
52. NKe26	17/018	575	muscle w shell	*n/a*	China		Negative		Inconclusive	37.75*	Negative
		577	muscle w shell	*n/a*	China		Negative		Negative	No Ct	Not tested
50. NKe24	17/018	580	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		581	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
60. NKe34	17/018	583	muscle w shell	*Litopenaeus vannamei*	Local and Imported Ingredients		Negative		Negative	No Ct	Not tested
		584	muscle w shell	*Litopenaeus vannamei*	Local and Imported Ingredients		Negative		Negative	No Ct	Not tested
56. NKe30	17/018	594	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		596	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
138. ML53	17/018	597	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		598	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested

153. ML68	17/018	603	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Inconclusive	37.68*	Negative
		604	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
154. ML69	17/018	612	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		613	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
155. ML70	17/018	616	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		617	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
152. ML67	17/018	621	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Inconclusive	37.66*	Negative
		622	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
151. ML66	17/018	633	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		634	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
141. ML56	17/018	639	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		640	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
143. ML58	17/018	645	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		646	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
116. ML31	17/018	650	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
		651	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
149. ML64	17/018	656	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		657	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
147. ML62	17/018	662	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested

		663	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
139. ML54	17/018	668	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		669	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
146. ML61	17/018	674	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		675	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
102. ML17	17/030	682	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		683	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
101. ML16	17/030	687	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
		688	muscle w shell	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
. NKe62	17/030	698	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		699	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML78	17/030	704	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		795	muscle w shell	*n/a*	n/a		Negative		Inconclusive	37.8*	Negative
ML80	17/030	710	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		712	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML77	17/030	722	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		723	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
Nke61	17/030	729	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		730	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested

ML79	17/030	736	muscle	*n/a*	n/a		Negative		Inconclusive	38.85*	Negative
		737	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML73	17/030	740	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		741	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML36*	17/030	746	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		747	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML76	17/030	752	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		753	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
126. ML41	17/030	764	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
		765	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
VMC3	17/030	770	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		771	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
58. NKe32	17/030	775	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		776	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
VMC1	17/030	783	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		784	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML75	17/030	787	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		788	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
VMC2	17/030	793	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested

		794	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
123. ML38		799	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		800	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
ML74	17/030	805	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		806	muscle w shell	*n/a*	n/a		Negative		Negative	No Ct	Not tested
ML81	17/030	813	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		814	muscle	*n/a*	n/a		Negative		Negative	No Ct	Not tested
115. ML30	17/030	822	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		823	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
VMC4	17/030	828	pleo/gill. 9 pools n=1	*n/a*	n/a		Negative		Negative	No Ct	Not tested
		829	pleo/gill. 9 pools n=1	*n/a*	n/a		Negative		Negative	No Ct	Not tested
156. ML71	17/018	837	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		838	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
157. ML72	17/018	843	muscle w shell	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
		844	muscle w shell	*Litopenaeus vannamei*	Vietnam		Negative		Negative	No Ct	Not tested
148. ML63	17/018	849	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		850	muscle w shell	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
144. ML59	17/018	856	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested

		857	muscle	*Litopenaeus vannamei*	Malaysia		Negative		Negative	No Ct	Not tested
145. ML60	17/018	863	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		864	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
125. ML40	17/018	867	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
		868	muscle	*n/a*	Vietnam		Negative		Negative	No Ct	Not tested
140. ML55	17/018	872	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		873	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
150. ML65	17/018	879	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
		880	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
142. ML57	17/018	885	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
		886	muscle	*n/a*	China		Negative		Negative	No Ct	Not tested
124. ML39	17/018	889	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
		890	muscle	*Litopenaeus vannamei*	China		Negative		Negative	No Ct	Not tested
39. NKe13	17/010	902	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested
		903	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested
40. NKe14	17/010	906	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested
		907	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested
32. NKe6	17/010	910	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested
		912	pleopod + gill	*Scylla serrata*	Thailand		Negative		Negative	No Ct	Not tested

27. NKe1	17/010	914	pleopod + gill	*Scylla serrata?*	Thailand		Negative		Negative	No Ct	Not tested
		915	pleopod + gill	*Scylla serrata?*	Thailand		Negative		Negative	No Ct	Not tested
84. AD2		920	muscle w shell	*n/a*	Malaysia		Negative		Negative	No Ct	Not tested
85. AD3		921	muscle w shell	*n/a*	China		Negative		Negative	No Ct	Not tested

Discussion

Appropriateness of tissue type

Prior to undertaking this testing, the available tissue type was assessed for its appropriateness. Given that significant volumes of this commodity have been demonstrated to be entering natural waterways within and adjacent to areas of commercial prawn fishing and prawn farming operations it was deemed necessary to assess the risk of this commodity for these pathogens.

Taura Syndrome Virus (TSV)

OIE target tissue for TSV testing is principally cuticular epithelium (or hypodermis) of the general exoskeleton, foregut, hindgut, gills and appendages, and often in the connective tissues, the haematopoietic tissues, the lymphoid organ (LO), and antennal gland. In chronic infections the LO is the principal target tissue. Given the cuticle/ subcuticular tissue virus localisation in TSV infected prawns the available tissue type was deemed appropriate for the study and representative of the objectives of the study given the commodity risk type. Acknowledging that a more sensitive survey for TSV would target lymphoid organ from whole prawns.

Yellow Head Virus genotype 1 (YHV-1)

OIE target tissue for YHV-1 testing is lymphoid organ, haemocytes, haematopoietic tissue, gill lamellae and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts and ganglia with surveillance ideally targeting the lymphoid organ (ideally) or gill. It was noted that this is not the ideal sample for the most sensitive surveillance for YHV, but still of value given this represents the bulk of the uncooked commodity type imported into Australia. It was considered to collect new tissue homogenates and nucleic acid extracts from the lymphoid organ and gill (pooled tissue types, pooled across 5 individuals) for the prawns that were submitted whole, but this only represented a small subset of the available survey samples and that dissecting the lymphoid organ would be difficult in the retail product.

Assessment of commodity risk

This study provides some confidence that the risk of YHV-1 and TSV presence in the untreated raw imported prawn commodities, tested in this study, is not high.

However, it cannot be concluded that the entry risk of YHV-1 and TSV associated with the entire imported prawn trade is negligible, given limitations associated with the study design and relatively small number of samples tested.

There is insufficient data from this study to infer whether the suspected trace amounts of TSV detected would be sufficient to provide an infective dose for prawns, or, for the many other carrier species identified (Overstreet, Jovonovich and Ma, 2009). While no real data are available concerning survival and resistance of TSV, it is generally considered as extremely resistant, particularly in seawater (Stentiford, Bonami and Alday-Sanz, 2009).

Historically, TSV infected shrimp have also been found in products sampled in US markets (Nunan et al., 2004) and were reported from Australia testing also. Viable YHV-1 has been detected previously in imported frozen prawn in Australia (McColl *et al.*, 2004).

Hence the present border controls, which do not require pre and at-border testing for TSV routinely, could allow entry of these commodities into Australia containing potentially infectious virions.

It was shown that TSV virions found in the faeces of sea birds after feeding on diseased shrimp were still infectious for up to 48 hours (Garza et al., 1997; Vanpatten et al., 2004). Such data indicates that should infected, imported, uncooked prawn commodities end up as recreational bait or berley, there is a ready

vector for transfer from wild fishery locations, onto nearby prawn farms. Once on farm, TSV will be difficult to contain, with birds providing a vector for horizontal transmission, within and between farms.

A study in Thailand reveals that mud crab (*Scylla serrata*) and other wild crab species (viz., *Uca vocans, Sesarma mederi*) can be experimentally infected with TSV by injection and by feeding with TSV infected shrimp carcasses. The resulting infections can persist for up to 50 days and thus crabs may act as carriers of TSV and can pose potential risk to shrimp aquaculture (Kiatpathomchai et al., 2008). However, natural infections of these species in wild caught crab were not found in the published literature.

Although not documented, vertical transmission may be important since Taura Syndrome is best known as a disease of nursery phase shrimp, occurring within 14 to 40 days of stocking into grow out ponds (Lightner, 1996). Oral transmission, particularly due to cannibalism or by contaminated water also occurs (Soto et al., 2003; White et al., 2002). An aquatic insect, the water boatman (*Trichocorixa reticulate*) feeding on shrimp carcasses in ponds was demonstrated to serve as a mechanical vector (Brock, 1997).

The infectious dose of TSV is not documented due to the lack of available crustacean cell lines or other types of cell that support the replication of shrimp viruses. In vivo titration has not been performed for TSV (Stentiford, Bonami and Alday-Sanz, 2009).

The generic process of cooking these commodities prior to entry into the Australian distribution network would further reduce risk for Australian prawn farming and commercial fishing sectors from entry of these exotic viruses and those pathogens for which this study did not include in its testing regime such as infectious myonecrosis virus (IMNV), acute hepatopancreatic necrosis disease (AHPND), Laem-Singh virus (LSNV) and *Enterocytozoon hepatopenaei* (EHP). The nature of prawn farming has seen a continual emergence of new infectious pathogens, predominantly from SE Asian sources.

The use of biosecurity principles is often spoken of to control the spread of infectious diseases. With the gold standard of biosecurity to keep exotic pathogens out of your country through installing measures which effectively mitigate the risks. Such measures in the long term are safer and cheaper than attempting to control diseases post-entry.

The trend of disease emergence in prawn farms is likely to continue, and an absence of highly effective border controls in Australia, such as cooking, to protect against known, and as yet, unknown or emerging pathogens will threaten the future productivity and expansion of the Australian prawn farming industry.

Conclusion

105 imported uncooked prawn commodities tested negative for the presence of YHV-1 by PCR.

15 of 105 imported uncooked prawn commodities gave an inconclusive, high CT score, reaction in testing for TSV by PCR. Re-tests of re-extractions from source pools of prawns were all negative.

It is suspected that very low levels of TSV may have been responsible for the test results.

Implications

The inconclusive results in 15 of 105 uncooked prawn commodities for TSV is a concern for the potential entry of a viable exotic prawn pathogen into Australia. The diversion of imported uncooked prawns for use as bait or berley by recreational anglers has been demonstrated as a risk pathway for release of pathogens should they be present within uncooked imported product.

The establishment of these exotic viruses would be profoundly detrimental for the prospects of the growing Australian Prawn Farming industry, and also potentially deleterious for wild capture crustacean industries.

The use of cooking as a sanitary measure for reduction of risk in imported prawn commodities could demonstrably reduce risk of viable pathogen entry into Australia, via this commodity trade.

Recommendations

Monitoring of the disease status of imported prawns at retail will remain important, to build confidence that the new enhanced Australian border control measures are being effective at preventing serious prawn pathogen entry.

Extension and Adoption

Results of testing have been distributed to the APFA Executive and R&D Committee.

Results will be discussed at the Annual APFA meeting.

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Appendix 1 – Project staff

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