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Supplementary report to: 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

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

Survey for White spot syndrome virus (WSSV) in retail seafood products: supplementary tests, 30-4-17

Background

Additional tests were requested for samples of retail seafood product reported to be of Australian origin where a positive result for the qPCR test for WSSV DNA was obtained. A procedure for surface decontamination and dissection of internal tissue was implemented. This was intended to help distinguish natural infection with WSSV from surface contamination with the virus that could occur at any time during processing, offer for sale and processing for the survey.

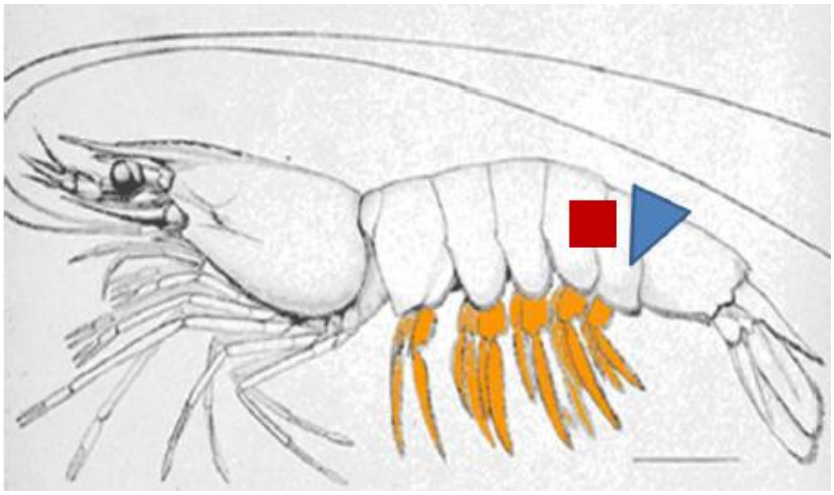
Outcome

The criteria for detection of WSSV DNA that was applied for demonstration of WSSV in some imported seafood product was satisfied for one of the five commodities identified for further testing (ML44). Laboratory data for the other retested samples was most consistent with the presence of WSSV DNA as a surface contaminant.

Method

Commodities identified for retesting each comprised 6 pools of 5 prawns that were thawed in ice. Surface disinfection was performed on individual shrimp using a freshly prepared 1% solution of Virkon-S (Antec). The surface of the shrimp (including the previously cut surfaces at the site of the initial sample) was saturated with a contact time of 1 minute before rinsing with sterile miilQ water.

A sample of 0.1 g of abdominal muscle was obtained immediately cranial to the previous sample using a sterile scalpel blade. All surface margins were excised. The location of the specimen is indicated in red in the schematic below. The original test specimen is indicated in blue for those samples in which pleopods were not available for testing.



Each sample was prepared for testing as previously reported: muscle tissue from each pool of 5 shrimp was homogenized by bead-beating with 1 ml of RLT buffer (Qiagen) and 5 µl Dx reagent (Qiagen). Nucleic acids were purified from an aliquot of tissue homogenate that represented 50 mg of pooled tissue using the All-for-One Vet Biosprint Kit (Qiagen). The Xeno DNA internal positive control system (Life Technologies) was used to test for inhibitors of PCR. The qPCR assay for WSSV DNA used the primers and probe described initially by East et al. (2004).

Results

Thirty six pools of 5 prawns were tested using the surface disinfection internal abdominal muscle sample procedure tissues for 1 commodity ID as a procedure control (Table 1) and 5 commodity IDs identified for further evaluation (Table 2). The qPCR for WSSV DNA was negative for all 6 pools prepared from 4/5 of these commodities. The commodity ID labeled ML44 produced 3/6 positive pool results. The Ct values were high, consistent with only a trace amount of WSSV DNA. Repeatable positive tests for WSSV qPCR were demonstrated for these 3 samples using a second preparation of nucleic acids from the surface disinfected, internal abdominal muscle tissue homogenates.

All positive and negative control samples for tissue homogenization, nucleic acid purification and qPCR provided expected results, including positive tests for the internal positive control (Xeno DNA).

Procedure control for surface disinfection and resampling after storage. The resampling procedure was performed on 6 pools of 5 prawns from Commodity ID NKe35. This sample of imported prawns was confirmed to be positive for WSSV DNA with a moderate-high viral load and had been subject to the same laboratory conditions as the samples for retest. The disinfection procedure did not prevent detection of WSSV in internal tissues, however, consistently higher Ct values indicate possible loss of sensitivity (Table 1). This would be expected due to one or more of the following: use of a specimen where WSSV is less abundant in natural infection; additional storage time and freeze-thaw cycle leading to degradation of the target viral nucleic acids; possible impact of the application of Virkon-S disinfectant on virus present in internal tissues.

Table 1: Retest of WSSV positive prawns to assess the impact of surface disinfection and additional

storage.

Commodity ID	SVC No.	Pool ID	Tissue submitted	qPCR, East assay (Ct)	
				Initial test: muscle and subcuticular epithelium	**Retests:** Internal muscle after surface disinfection
NKe35	17/010	379	Muscle, tail on	25.70	27.74
	17/010	380	Muscle, tail on	26.00	28.88
	17/010	381	Muscle, tail on	24.70	27.51
	17/010	382	Muscle, tail on	25.10	26.55
	17/010	383	Muscle, tail on	25.40	25.72
	17/010	384	Muscle, tail on	25.80	28.19

Tests to increase the sensitivity of detection of WSSV DNA in trace positive samples. Additional tests on individual prawns were undertaken for commodity IDs in which there were positive results by qPCR but no confirmatory conventional PCR positive results to provide confirmation (Pools 454, 820 and 821; n=15 individual prawn samples). Individual tissue homogenates were prepared prior to surface disinfection for all prawns in these groups (n=15). If the prevalence was low this would identify a stronger positive from which conventional PCR and sequence analysis could be used to evaluate the nature of WSSV positive result with a molecular epidemiological approach. For example, viral sequence from these commodities might be from a variant virus, explaining the inconsistent results or a match might be made with unique WSSV sequence in product in which it had come into contact during processing and retail. All of the individual samples tested negative using the East WSSV qPCR assay.

***Notes on the use of Virkon-s for surface disinfection*.** Virkon is an oxidative disinfectant prepared in combination with detergents to potentiate microbial decontamination. The product is empirically predicted to inactivate WSSV and efficiently degrade DNA so that inactivated virus would not be detected by qPCR. Virkon-S is not registered for the purpose described here and there is no guidance on the penetration of Virkon-S into animal tissue. The contact time used is shorter than would be applied in disinfection of solid surfaces and equipment because the load of virus needing disinfection was known to be very low and the risk of degrading WSSV in internal tissues was to be avoided. This was considered to be the best empiric option for exclusion of external surface contamination with WSSV DNA without compromising the sensitivity of the test of internal tissues.

The procedure control samples indicate the potential loss of sensitivity as a consequence of using Virkon-S which might have been enough to create a false negative result in prawns with a true trace natural infection. Alternatively, the positive result for surface disinfected tissues might still represent surface contamination and inadequate activity of the disinfectant. There is evidence that recommended disinfection protocols including Virkon can be inadequate in the presence of high organic load as would be present in this application (Aiello, et al., 2016; Hick, et al., 2015).

References

Aiello, R., Zecchin, B., Tiozzo Caenazzo, S., Cattoli, G., De Benedictis, P., 2016. Disinfection protocols for necropsy equipment in rabies laboratories: Safety of personnel and diagnostic outcome. Journal of Virological Methods. 234, 75-79.

East, I.J., Black, P.F., McColl, K.A., Hodgson, R.A.J., Bernoth, E.M., 2004. Survey for the presence of White Spot Syndrome virus in Australian crustaceans. Australian Veterinary Journal. 82, 236-240.

Hick, P., Evans, O., Looi, R., English, C., Whittington, R.J., 2016. Stability of Ostreid herpesvirus-1 (OsHV-1) and assessment of disinfection of seawater and oyster tissues using a bioassay. Aquaculture. 450, 412-421.

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Table 2. WSSV qPCR results including tests of internal muscle tissue after surface disinfection for pools of 5 prawns from selected commodities.

Commodity ID	SVC No.	Pool ID	Tissue submitted	Previous results			Retest: Internal tissue after surface disinfection		
				Screening qPCR (East assay)		Conventional nested PCR	Screening qPCR (East assay)		
				Specimen	Result (Ct)		Specimen	Result (Ct)	Repeat test [a.]
NKe 5	17/010	451	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	452	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	453	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	454	Whole prawn	pleopods	**39.99**	Negative	Internal abdominal muscle	No Ct	
	17/010	455	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	456	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
NKe7	17/010	240	muscle + some shell	muscle + subcuticular epithelium	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	241	muscle + some shell	muscle + subcuticular epithelium	39.3	Not tested	Internal abdominal muscle	No Ct	
	17/010	242	muscle + some shell	muscle + subcuticular epithelium	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/010	243	muscle + some shell	muscle + subcuticular epithelium	38.5	Negative	Internal abdominal muscle	No Ct	
	17/010	244	muscle + some shell	muscle + subcuticular epithelium	39.6	Not tested	Internal abdominal muscle	No Ct	
	17/010	245	muscle + some shell	muscle + subcuticular epithelium	39.3	Not tested	Internal abdominal muscle	No Ct	
NKe 59	17/030	816	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/030	817	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/030	818	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/030	819	Whole prawn	pleopods	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/030	820	Whole prawn	pleopods	38.9	Not tested	Internal abdominal muscle	No Ct	
	17/030	821	Whole prawn	pleopods	38.7	Trace positive	Internal abdominal muscle	No Ct	

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Table 2, continued.

Commodity ID	SVC No.	Pool ID	Tissue submitted	Previous results			Retest: Internal tissue after surface disinfection		
				Screening qPCR (East assay)		Conventional nested PCR	Screening qPCR (East assay)		
				Specimen	Result (Ct)		Specimen	Result (Ct)	Repeat test [a.]
ML43	17/008	114	Peeled, no tail, shell	muscle	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/008	115	Peeled, no tail, shell	muscle	No Ct	Negative	Internal abdominal muscle	No Ct	
	17/008	116	Peeled, no tail, shell	muscle	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/008	117	Peeled, no tail, shell	muscle	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/008	118	Peeled, no tail, shell	muscle	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/008	119	Peeled, no tail, shell	muscle	36.94	Not tested	Internal abdominal muscle	No Ct	
ML44	17/008	96	Peeled, no tail, shell	muscle	No Ct	Not tested	Internal abdominal muscle	No Ct	
	17/008	97	Peeled, no tail, shell	muscle	32.22	Positive	Internal abdominal muscle	35.65	34.8
	17/008	98	Peeled, no tail, shell	muscle	37.52	Not tested	Internal abdominal muscle	38.68	36.15
	17/008	99	Peeled, no tail, shell	muscle	35.43	Not tested	Internal abdominal muscle	no Ct	
	17/008	100	Peeled, no tail, shell	muscle	34.55	Not tested	Internal abdominal muscle	35.78	35.45
	17/008	101	Peeled, no tail, shell	muscle	34.88	Not tested	Internal abdominal muscle	no Ct	

a. qPCR result for a second nucleic acid preparation extracted from the surface disinfected internal abdominal muscle tissue homogenates that were positive on initial retest.