Detection of White Spot Syndrome virus and Yellowhead virus in prawns imported into Australia

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Objective To determine whether viable White Spot Syndrome virus (WSSV) or Yellowhead virus (YHV) were present in prawn products imported into Australia.

Procedure A sample of fourteen uncooked prawns was obtained from a consignment imported from southeast Asia. Each of the prawns was examined for WSSV by polymerase chain reaction (PCR), and then a bioassay was conducted in which a 10% homogenate of cuticular epithelium from each of the prawns was inoculated intramuscularly into healthy challenge prawns (*Penaeus monodon*) from Australia. The latter were then monitored for clinical signs of disease, and tissue samples were processed for electron microscopy, histological examination and for detection of WSSV by in situ hybridization (ISH) using a commercial kit. Limited numbers of haemolymph samples from inoculated challenge prawns were also examined by PCR for the presence of WSSV and YHV. All work was carried out under microbiologically secure conditions.

Results Results of the initial PCR examination for WSSV on the imported prawns were not definitive. However, in the bioassay, several of the challenge prawns inoculated with homogenates from the imported prawns showed clinical signs of disease (inappetence and lethargy) within 24 h post inoculation (pi) and died at 1 to 4 days pi. Tissue samples from a number of moribund prawns demonstrated lesions typical of White Spot Disease (WSD), and the presence of the virus was confirmed by electron microscopy, ISH and PCR. YHV was also demonstrated by PCR in two challenge prawns inoculated with homogenates.

Conclusion Viable WSSV and YHV were present in frozen prawn products imported into Australia for human consumption from southeast Asia. Importation of frozen infected products may present a risk of transferring virus to wild and farmed populations of crustaceans in this country. To date, WSD and Yellowhead Disease remain exotic to Australia.
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Australia is considered to be free of WSD and YHD,[1] two of the most important viral diseases of farmed prawns (although neither is of concern to human health). WSD emerged in prawns in Taiwan in the early 1990s, and, over the past decade, the disease has spread throughout the prawn-producing countries of Asia.[2] More recently, it has also been found in the Americas where all major prawn-farming countries have been affected.[3] The annual global economic impact of WSD has been estimated at US$3 billion.[4] The disease may be associated with clinical signs such as the development of white spots in the cuticle, a general red discolouration of the body, and cessation of feeding.[5,6] It is also characterised by the rapid onset of high mortality with up to 90% of prawns dying within 2 to 4 days of the outbreak of disease. WSD is caused by WSSV, a large, enveloped, bacilliform double-stranded DNA virus that morphologically resembles a baculovirus, but which has now been proposed as the type species of the new family *Whispoviridae*.[7] Preliminary diagnosis of the disease is achieved by histopathological examination with a definitive diagnosis made by ISH using WSSV-specific nucleic acid probes. PCR may also be used to confirm a tentative diagnosis.[8]

YHD is also an economically important disease of farmed prawns because, like WSD, it is usually associated with extensive mortality and severe production losses.[9,10] It is caused by YHV, a single-stranded RNA virus that, it is proposed, belongs to the family *Coronaviridae*.[11] A PCR has also been developed for its detection.[8] In Australia, two yellowhead-like viruses have been described –LOV, an avirulent virus found in the lymphoid organ of healthy *Penaeus monodon*,[12] and GAV, which resembles YHV morphologically, and which causes mortality in *P monodon* with lesions that resemble those caused by YHV.[13] LOV and GAV are now recognised as isolates of the same virus.[14]

Over the past decade, up to about 10,000 tons of fresh chilled or frozen prawns have been imported into Australia per annum, mainly from southeast Asia. Yet, despite the fact that most of the imports originated from countries where WSSV and YHV are enzootic,[15] there have been no outbreaks of WSD and YHD in Australian farmed prawns. It is well-established that international trade of live aquatic species poses a great risk of disease spread. In addition, there is some evidence for the spread of prawn diseases such as WSD and YHD from enzootic regions to previously disease-free areas through the movement of frozen commodity shrimp.[3,16]

In order to determine whether viable virus is entering Australia via imported product from southeast Asia, a bioassay, using farmed *Penaeus monodon* from Queensland, Australia, was undertaken. Given the lack of prawn cell lines to attempt culture of prawn viruses,[17] a bioassay is the only way to detect the presence of viable prawn viruses. While this investigation was particularly

AAHL	Australian Animal Health Laboratory
GAV	gill-associated virus
H&E	haematoxylin and eosin
IB	inclusion bodies
ISH	in situ hybridization
LOV	lymphoid organ virus
PCR	polymerase chain reaction
PBS	phosphate buffered saline
pi	post inoculation
WSD	White spot disease
WSSV	White spot syndrome virus
YHD	Yellowhead disease
YHV	Yellowhead virus

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concerned with WSSV, the nature of the exercise also allowed an examination of the imported prawns for the presence of viable YHV.

Materials and methods

Preparation of inocula

Fourteen prawns from a batch that had been imported into Queensland from southeast Asia were examined. The tails from these prawns were each processed as a separate test sample. Cuticular epithelium dissected from each tail was homogenised using a mortar and pestle, resuspended in a minimum volume of PBS (pH 7.4) and centrifuged (2000 *g* for 10 min). The supernatant fluid from each sample was then decanted and, with PBS, made up to a volume equivalent to 10 times the volume of the pelleted tissue. This 10% suspension was then filtered (0.45 µm), and held at -80°C, ready for inoculation.

Table 1. Experimental protocol for the bioassay identifying room, tank, treatment and number of challenge prawns/tank/treatment.

Tank	Inoculum	Number of prawns/tank
Room C8: Negative Controls		
1, 2	Normal prawn haemolymph diluted 1/1000	4
3, 4	Normal prawn head or tail homogenate diluted 1/10	4
5, 6	PBS	4
Room C6: Positive Controls		
A, B	Haemolymph (diluted 1/1000) from WSSV-infected prawn	4
C, D	Haemolymph (diluted 1/10000) from WSSV-infected prawn	4
E, F	Head homogenate (diluted 1/10) from WSSV-infected prawn	4
G, H	Tail homogenate (diluted 1/10) from WSSV-infected prawn	4
I	Haemolymph (diluted 1/1000) from WSSV-infected prawn	8
J, K	Haemolymph (diluted 1/10000) from WSSV-infected prawn	5, 7
Room C7: Test Samples		
M	Tail homogenate #1 (diluted 1/10)	5
N to Z	Tail homogenates #2 to #14 (each diluted 1/10)	4

Four types of negative control inocula were prepared: (1) One of the Australian farmed prawns, supplied for use as challenge animals in the bioassay, was used as a source of cuticular epithelium. The prawn was killed, the epithelium dissected from the tail, and the inoculum prepared as described above; (2) the soft tissues of the head/thoracic region from the same prawn were homogenised and treated as outlined for the tail homogenates; (3) Haemolymph samples obtained from other normal Australian prawns; and, (4) PBS only.

Homogenates of the cuticular epithelium from the tail, and of the soft tissues from the head/thoracic region, of prawns experimentally infected with WSSV (provided by T Flegel, Bangkok, Thailand) were used as positive control inocula. Haemolymph samples from similarly infected prawns were also used.

Experimental protocol for the bioassay

The challenge prawns, *Penaeus monodon*, were obtained from a Queensland farm (Gold Coast Marine Aquaculture) and housed in the microbiologically-secure AAHL. Prawns were maintained in 100 µm-filtered sea-water obtained from the Marine and Freshwater Research Institute, Queenscliff, Victoria with 50% (v/v) of the water changed on alternate days. Water temperature was maintained at 23 to 24°C. Each of the three main experimental groups of prawns (negative controls, positive controls and test samples) were held in separate rooms to ensure that cross-contamination did not occur. Tanks, each containing at least four challenge prawns per 100 L of sea-water, were set-up for each treatment group (Table 1); there were duplicate tanks for some of the controls. All prawns received 100 µL of the appropriate inoculum intramuscularly into the tail, and the experiment was terminated at 5 days pi.

Sampling

Following inoculation, all experimental groups were monitored, twice daily, for clinical signs. As soon as dead prawns, or prawns with abnormal clinical signs (cessation of feeding; lethargy), were observed in a group, samples (haemolymph, gills, head/thorax and tail) were collected from the remaining prawns in that group, and processed for laboratory examination. Prawns were killed using standard methods.[18]

Sample processing

A syringe and needle were used to collect, into sodium citrate, a sample of haemolymph from the major ventral sinus of each live prawn. Samples were then stored at -80°C. Haemolymph was examined by electron microscopy for the presence of virus particles. The head/thoracic samples were fixed in Davidson's fixative for 24 h, and then transferred to alcohol, pending routine processing for histological examination. Sections were either stained with H&E, or prepared for ISH.

In situ hybridisation

ISH to detect WSSV in infected tissue was performed using a commercial kit (DiagXotics Inc., Wilton CT, USA). The method was essentially that recommended by the distributor, although hybridisation of the probe was overnight at 42°C. Tissues from a single challenge prawn from each of the following experimental groups were tested: (1) tank 1 (negative control); (2) tank D (positive control); (3) tanks O, R, W and Z (each inoculated with different test samples). A similar kit was used to attempt detection of YHV in prawn tissues (DiagXotics Inc., Wilton CT, USA).

Polymerase chain reactions

A small piece of the tail (approximately 100 mg) was collected from each of the 14 imported prawns. Each piece, consisting of chitin, epidermis and underlying muscle, was homogenised individually with a disposable pestle in an Eppendorf tube. Nucleic acid was then extracted, using standard phenol-chloroform extraction procedures and ethanol precipitation. The WSSV PCR was performed on each sample (see below).

Single challenge prawns from Tanks 3, A, G, N, R, V and W in the bioassay were also examined. Haemolymph was collected from each prawn into anticoagulant. The samples were centrifuged (13,000 *g* for 1 min) to pellet cells, and nucleic acid was then extracted from the supernatant fluid (QIAamp Viral RNA Mini Kit; Qiagen Pty Ltd, Clifton Hill, Victoria. This kit is also recommended by the manufacturer for the extraction of nucleic acid from DNA viruses). Primers and procedures for the WSSV and YHV PCRs were those recommended by the OIE[8] with a few slight modifications to conform to standard buffers

and cycle times that are used in this laboratory. Extraction of nucleic acid and all PCRs were conducted in a dedicated PCR Suite, the latter established to avoid potential contamination problems. A WSSV recombinant plasmid (R Hodgson, unpublished), and partially purified YHV (T Flegel, Thailand) were used as positive controls.

Sequencing

Purified YHV-specific PCR products were sequenced using fluorescence-based dideoxy BigDye terminator chemistry (Applied Biosystems, Scoresby, Victoria 3179), and the sequencing products were analysed using an ABI PRISM 377 DNA Sequencer (Applied Biosystems). Sequence data were managed using BioManager (Australian National Genomic Information Service, www.angis.org.au), and homology searches were conducted using the BLAST server at the National Centre for Biotechnology Information, USA.

Table 2. Presence of WSSV-like intranuclear IBs in prawns inoculated with test samples.

Tank	Sex	Cuticular/gut epithelium	Gills	Antennal gland epithelium	Lymphoid Organ	Haematopoietic Tissue	Heart
M	M	-	-	-	-	-	-
N	M	-	-	-	-	-	-
O	M	4+	1+	2+	NE	2+	1+
P	F	2+	1+	1+	NE	NE	1+
Q	M	3+	2+	1+	2+	NE	1+
R	F	3+	1+	1+	2+	1+	1+
S	F	4+	1+	2+	1+	1+	1+
T	ND						
U	F	4+	NE	1+	2+	2+	1+
V	F	2+	1+	2+	NE	-	-
W	F	3+	1+	2+	2-3+	2+	-
X	F	-	-	-	-	-	-
Y	F	-	-	-	NE	-	-
Z	F	3+	-	2+	1+	-	1+

NE: not examined because this tissue was not present in available section
-: no IBs observed
1+ to 4+: occasional to numerous IBs
ND: no tissues available
IBs were also noted in the vas deferens of one challenge prawn in the bioassay

Electron microscopy

Samples of cuticular epithelium from the challenge prawns were submitted in 70% (v/v) ethanol. They were examined by transmission electron microscopy, together with tissues from known WSSV infected and normal animals (positive and negative controls, respectively). All samples were examined in a Philips CM120 transmission electron microscope at 120 kV.

Results

Clinical signs and pathology

Dead or moribund challenge prawns were found in all 14 of the treatment groups (tanks M to Z) over the 5-day course of the bioassay. In total, 47 of the 57 prawns inoculated with test samples either died and were discarded or became moribund. The latter, which were characterised by a loss of appetite and lethargy, were killed, and samples were collected for laboratory examination. Mortality occurred in positive control groups as early as 1 day pi, and, over the course of 5 days, all positive control prawns either died or became moribund. There were no abnormal clinical signs, or deaths, in prawns from five of the six negative control groups; in the remaining group, two of four prawns died at 5 days pi. Histological examination of the remaining prawns in this tank, and of other negative control prawns that were sacrificed, revealed no obvious lesions; in particular there was no indication of a viral infection. It is likely that the two deaths were due to non-specific causes.

Histological examination of tissues from moribund prawns that had been inoculated with either test samples (Table 2) or with positive control virus revealed lesions consistent with infection by WSSV (Figures 1, 2A). In particular, intranuclear eosinophilic to basophilic inclusion bodies were observed in many tissues including: subcuticular epithelial cells of the exoskeleton and the stomach; antennal gland epithelium; sheath cells in the lymphoid organ; haematopoietic cells; fixed macrophages of the heart; and, least frequently, in the vas deferens.

Whereas there were no lesions that were pathognomonic of YHV infection in prawns inoculated with the test samples, multifocal to diffuse necrosis of the intertubular connective tissue of the hepatopancreas in a number of these prawns suggested that YHV could not be excluded as an aetiological agent.

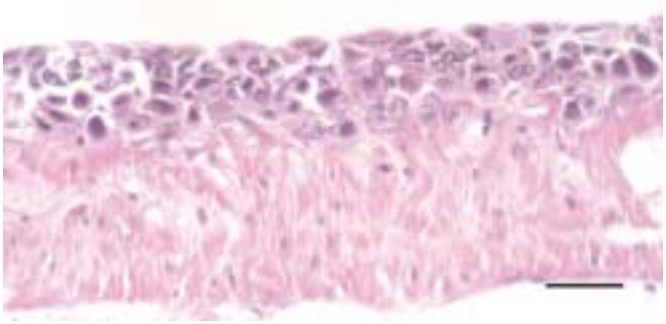

Figure 1. Sub-cuticular epithelium from a WSSV-infected challenge prawn. The majority of epithelial cells contain prominent intranuclear IBs. H&E. Bar represents 67 µm.

In situ hybridisation

Using the WSSV ISH kit, positive staining, consistent with the positive control (provided in the kit), was observed in all samples that were examined (Figure 2B), except for the negative control tissues. In general, the location of staining corresponded with the presence of inclusion bodies in cells. No specific staining was apparent in the samples tested with the YHV ISH kit. However, the positive control (provided with the kit) also lacked convincing staining.

PCRs and sequencing

Fourteen of the imported prawns were originally tested for WSSV in Queensland by PCR (R Hodgson, pers comm). Although some of these were found to be positive, when testing of the original 14

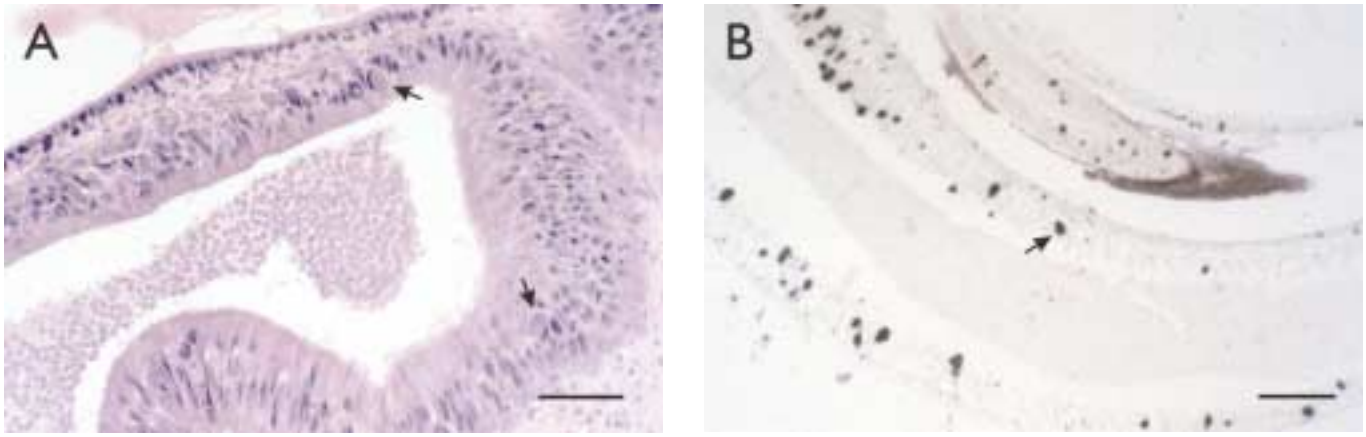

Figure 2. Vas deferens from a WSSV-infected challenge prawn. (A) The intranuclear viral IBs are visible (arrows). H&E. Bar represents 30 µm. (B) The IBs are clearly localised by their reaction with a labelled WSSV-specific DNA probe (arrow). ISH. Bar represents 70 µm.

prawns was repeated at AAHL, only one was weakly positive, and two others produced equivocal results (results not shown). These samples were not examined for YHV.

PCR results for the bioassay are summarized in Table 3. WSSV PCRs conducted on the WSSV plasmid and on haemolymph from single prawns out of Tanks A and G, each containing positive control WSSV-inoculated prawns, yielded, in each case, a product of the expected size for the primary and the nested WSSV PCR. There was no product in a negative control prawn, or in a sample of water. Specific WSSV PCR products were also detected in primary and nested PCRs for three of the four test samples that were examined (Tanks R, V and W). The sample that was negative by PCR (Tank N) was also negative by histopathology. It was not tested by ISH.

A YHV PCR yielded a product of the expected size for the YHV positive control virus, and for haemolymph samples from two of the test samples that were examined (Tanks R and W). The PCR product from one of the test samples (Tank W) was sequenced, and a homology search, using the BLAST program, demonstrated 100% identity to the putative RNA polymerase gene of YHV (nt 1070 to 1137). There were no YHV PCR products in uninoculated, or WSSV-infected, Australian prawns in Tanks 3, A and G.

Electron microscopy

Viruses consistent with the size and morphology of WSSV were observed within sections of the cuticular epithelium of the challenge prawns that were examined. The viruses were about 296 to 304 nm in length and 77 to 88 nm in diameter (Figure 3A) and were located in the remnants of nuclei. Viruses were observed with either single or double envelopes and possessed an electron dense nucleoid. Within the matrix surrounding many of the viruses there were parallel strands of putative nucleocapsids of about 10 nm in diameter. These structures correspond to the 10 nm globular structures that constitute the strands of nucleocapsids of WSSV. The poor ultrastructure was attributed to the tissues being originally collected into Davidson's fixative.

Positive control WSSV-infected prawns contained viruses that morphologically resembled baculoviruses (Figure 3B). Such structures were not observed in the negative controls. The viruses observed in the positive control were indistinguishable from those identified within the epithelium of the challenge prawns.

Table 3. Detection of WSSV and YHV by PCR on haemolymph samples from selected prawns.

Sample	WSSV		YHV
	Primary	Nested	
Controls			
Tank 3, negative control prawns	-	-	-
Tank A, WSSV positive control prawns	+	+	-
Tank G, WSSV positive control prawns	+	+	-
Water, negative control	-	-	-
WSSV plasmid	+	+	-
YHV positive control	-	-	+
Test samples			
Tank N, test sample # 2	-	-	-
Tank R, test sample # 6	+	+	+
Tank V, test sample # 10	+	+	-
Tank W, test sample # 11	+	+	+

Discussion

The results presented here have demonstrated that viable WSSV and YHV, two prawn viruses that are exotic to Australia, were present in a batch of frozen prawns imported into Australia from southeast Asia. Whereas a WSSV PCR conducted on 14 of these prawns in Queensland suggested the presence of WSSV in a few of them, the results of the OIE WSSV PCRs, conducted later at AAHL on these initial samples, were not definitive. However, subsequent results from the bioassay provided unambiguous evidence for the replication of not only WSSV but also YHV in the challenge prawns. The evidence included: clinical signs of disease and death in a high proportion of WSSV- and YHV-free Australian challenge prawns inoculated with samples prepared from the imported prawns; histological lesions characteristic of WSSV and, in some cases, suggestive of YHV in affected challenge prawns; WSSV-like particles in affected challenge prawns by electron microscopy; a positive response in the affected challenge prawns with the WSSV ISH kit demonstrating a clear association between the histological lesions and the presence of WSSV; and specific WSSV and/or YHV PCR products of the expected size in a number of the challenge prawns that were examined. Sequencing of one of the putative YHV PCR products demon-

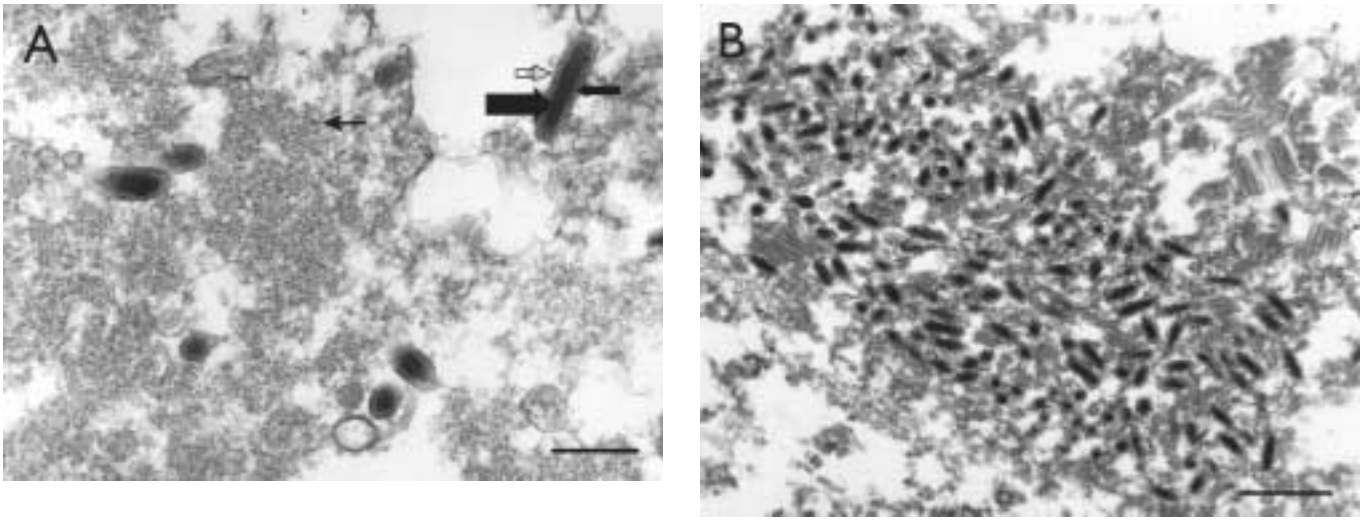

Figure 3. Transmission electron micrographs of WSSV within WSSV-infected challenge prawn (A) and positive-control (B) samples. (A) WSSV within nuclear remnants illustrating the baculovirus shape (arrow) of the viruses with envelopes (open arrow) and electron dense cores (large arrows). The 10 nm nucleocapsids are present between the viruses (thin arrow). (B) WSSV in control samples. Structures present in (A) are also apparent in this sample although there are more nucleocapsids in the challenge prawns. Bar represents 200 nm (A) and 500 nm (B).

strated that neither LOV nor GAV, now recognised as a single Australian YHV-like virus,[14] had caused a false-positive result in the PCR.

The only apparent contradiction in the results occurred with Tank N, where four prawns were inoculated with a tail homogenate from an imported prawn. Histological examination of some of the challenge prawns, and a PCR on haemolymph from one prawn from this tank, were negative for WSSV. However, ISH on another prawn from this tank was positive. This may simply suggest that not all challenge prawns in the tank had become infected by WSSV by the time they were examined.

In view of the range of evidence supporting the presence of WSSV (and YHV) in the imported prawns, the inability to obtain a definitive WSSV diagnosis on the imported samples when they first arrived at AAHL requires an explanation. Instead of collecting just cuticular epithelium from each of the imported prawns, the samples first collected for testing by PCR consisted mainly of muscle with a small piece of overlying cuticular epithelium. As it is only the latter that would harbour a significant amount of virus in such a sample, it is likely that any viral DNA in each sample was overwhelmingly diluted by the cellular nucleic acid, making detection difficult. Subsequent re-examination of cuticular epithelial samples from the imported prawns by a real-time PCR for WSSV has in fact demonstrated the presence of WSSV in all 14 imported prawns (KA McColl, unpublished).

The results of the current study suggest that not only was there genetic material from two exotic viruses in some prawns imported into Australia, but also that, in some cases, viable viruses were present in the imported prawns. Such viruses have been shown to be capable of causing disease in Australian prawns (as demonstrated by the results in the bioassay prawns), although it should be recognised that, in the current study, challenge prawns were inoculated intramuscularly with an homogenate of cuticular epithelium from the imported prawns rather than exposed via a natural route of infection. The results of the current work are consistent with observations from previous studies, which have shown that frozen prawns imported into the USA contained viable exotic viruses capable of causing disease when experimentally transferred to susceptible penaeid prawns.[3,16] One of these studies also demonstrated some transmission of WSSV per os in challenge prawns.[3]

The imported prawns used in the current experiment had been frozen and thawed several times indicating that both WSSV and YHV in prawn tissues are particularly resistant to this treatment. Indeed, tissue extracts and haemolymph from WSSV-infected prawns, stored frozen at AAHL for several years, remain a reliable source of infective virus (KA McColl et al, personal observations). Thus it is not unexpected that viable WSSV is present in frozen prawns exported for human consumption from regions where WSD is enzootic. This is of particular concern to Australia and other WSD- and YHD-free countries that import frozen prawns. Such countries need to assess, and manage, the risks associated with the international movement of frozen prawns.

Prawns infected with WSSV or YHV would still be fit for human consumption since these viruses cause no known human health problems. However, several pathways have been identified by which imported and infected prawns could become a source of infection to farmed and wild decapod crustaceans. Lightner et al[19] identified some of these pathways, which include: waste product, from reprocessing imported prawns, entering waters containing susceptible hosts; disposal of solid waste; use as bait; and, use as fresh food for aquatic animals. The situation is further complicated by the broad host range of WSSV, which is known to be capable of infecting not only penaeid prawns but also a number of other decapod crustaceans including crabs, lobsters and some other species of prawns.[20,21] If the quality of a shipment of imported, infected prawns deteriorated (for any reason) such that they were no longer fit for human consumption, it is conceivable that their disposal, or use in other inappropriate ways, could indeed represent a threat to animal biosecurity. Despite the continued importation of fresh, frozen prawns from regions where WSD and YHD are enzootic, a recent study failed to produce evidence for the presence of these devastating diseases in Australia.[1]

Acknowledgments

The fourteen prawns imported into Queensland and the WSSV recombinant plasmid were kindly provided by Dr Richard Hodgson (CSIRO Livestock Industries, Longpocket Laboratory, Qld). Dr Tim Flegel (Mahidol University, Bangkok, Thailand) generously supplied the WSSV and YHV. GJ received an Overseas Fellowship from FAO of the United Nations, and he was also the grateful recipient of financial assistance from the Government of India and from Tamil Nadu Veterinary and Animal Sciences University. Lynette Williams and Tony Pye, both at AAHL, assisted with maintenance of the prawns and provided the automatic sequencing service, respectively. Frank Filippi, AAHL, prepared the photographs for publication.

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Distinct molecular phenotypes in bovine prion diseases

French scientists have described a distinct molecular phenotype of the BSE prion in three cattle. The cattle were among routinely diagnosed BSE cases detected during active surveillance using rapid tests at slaughterhouses and rendering plants. No clinical signs suggestive of BSE had been reported during their life. This finding was unexpected because phenotyping studies in mice (lesion profiles and incubation period) and in vitro (western blots on proteinase K digested prion protein) suggest that BSE is caused by a single strain which is uniform and stable, including following its transmission to other species.

The agent from these three cases is distinguished by a higher molecular mass of unglycosylated PrP^{res} and strong labelling with the P4 monoclonal antibody. PrP^{res} from previously described cases of BSE is characterised by predominance of the diglycosylated form in western blots and by weak binding with P4 monoclonal antibody.

Possible reasons for this unexpected finding are discussed.

Firstly, it may be that differences in the prion genes between these three cattle and the general cattle population could give rise to such variants. This is known to occur in human cases of sporadic CJD. However, sequencing of the entire open reading frame of the prion genes of two of the atypical cases and comparison with the general cattle population excluded this hypothesis.

Secondly, it has been shown in human CJD that distinct PrP^{res} from sporadic CJD and vCJD can be interconverted by altering their metal ion occupancy. This could not be demonstrated with these cattle BSE cases. Treatment with EDTA in the range of concentrations shown to modify the profiles of human PrP^{res} did not alter the profiles from either the typical or atypical bovine cases.

Thirdly, it may be that cattle have been infected by another source of the infectious agent, such as scrapie from sheep or goats. In earlier studies, when cattle were experimentally infected with a British natural sheep scrapie source, similar differences in profiles of the PrP^{res} were found.

A final suggestion to explain the findings in this report is that these three cattle cases may represent examples of the bovine equivalent of human sporadic CJD. That is, they have arisen as sporadic events in the cattle population and not as a result of foodborne infection. Such a rare event, followed by recycling via the food chain, has been suggested previously as one possibility for the origin of the BSE epidemic in the UK. The PrP^{res} profile with the sporadic form of human CJD differs from that of vCJD, which is associated with presumed human infection with the agent of BSE.

The authors of this report indicate that further studies are required to determine the frequency of novel phenotypes in cattle and also to biologically characterise them. However, they do suggest that their results reinforce the possibility that BSE has different forms, some of which may be hardly recognised when transmitted to different species. It may be that if different forms of the disease affect cattle then some cases of BSE could continue to be detected beyond any possibility of contamination by infected meat-and-bone meal.

A recent report from Japan indicates that an atypical form of the BSE agent was associated with cases of disease in Japan. Any similarity between the Japanese atypical form and that reported from France remains to be established.

Biacabe A-E, Laplanche J-L, Ryder S, Baron T. *EMBO Reports* 2004;5:110-115.