The Special Danger of Viral Pathogens in Shrimp Translocated for Aquaculture

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ABSTRACT: It is well known that the majority of invasive aquatic species have been established in new habitats as a result of intentional importation for aquaculture or recreational purposes. This includes shrimp and other crustaceans. As a result of unexpected difficulties that arose from some of these importations, the International Council for Exploration of the Seas (ICES) proposed a set of precautionary guidelines to follow to reduce risks associated with translocations. Briefly, these include preparation of a proposal to the ICES indicating the purpose and area of transfer and detailed biology of the transfer species, and preparation of a detailed impact analysis including environmental disease and socioeconomic issues. In the event that a decision is made to proceed with the introduction, a number of precautionary actions are recommended. For fish, it is recommended that eggs or fry be imported to strict quarantine (including disinfected effluents) and monitored until reared to broodstock size for freedom from disease, before a preliminary phase of limited distribution of their offspring to aquaculture facilities. If broodstock are imported, it is recommended that they be destroyed after spawning (for detailed pathogen assay) and that the offspring be raised and used similarly to the above. After one year of trouble-free, limited field tests, wider use or introduction to open waters may be initiated. For species that are part of commercial practice, abbreviated guidelines are recommended that include export and quarantine procedures. These guidelines are appropriate for vertebrate species (from fish to mammals), but not for shrimp, other crustaceans and arthropods in general, especially with respect to viral pathogens. This is because grossly healthy arthropods tend to carry cryptic viruses (possibly unknown) that can jump to endemic arthropod species and cause massive mortalities. This phenomenon has resulted in 3 major shrimp epizootics that have caused economic losses in the order of several billion US$ since the early 1990's. To avoid future repetition of this scenario, it is strongly recommended that the ICES guidelines be specifically modified for crustacean introductions to include, during the quarantine period, mandatory co-habitation studies with economically or ecologically important native crustaceans.

KEYWORDS: shrimp, translocation, cryptic, virus, epidemic.

INTRODUCTION

I contend that the greatest risk for spread of diseases lies by far with the careless, cross-boundary movement of living animals or fry destined directly for aquaculture facilities. In support of this, Food and Agricultural Organization (FAO) statistics (http://www.fao.org/waicent/faoinfo/fishery/statist/fisoft/dias/statisti.htm) show that about 65% of exotic species introductions have been intentional, and that 69% of these (39% of the total) have been for aquaculture. The vast majority of introduced species (82%) have been finfish, followed distantly by mollusks (9%) and crustaceans (6%). All the translocated animals would have had the potential to carry pathogens. The most ominous would be viral and parasitic diseases that would otherwise have very little chance of being translocated.

Because of growing perception of environmental and disease threats associated with translocation of living marine animals and plants, the International Council for the Exploration of the Seas (ICES) in 1973 and 1994 introduced guidelines for reducing the risk of pathogen introduction. Briefly, they recommended that an imported broodstock population should be established in an approved quarantine facility. The progeny, but not the original import, should be transplanted into the natural environment or farms, only if no diseases or parasites became evident during the quarantine period. The quarantine period would provide opportunity for observation, and its duration would be at least one complete life cycle, regardless of the stage at which the shrimp had been introduced. All effluents from the quarantine facilities would be disinfected in an approved manner, killing all living organisms. If evidence of disease was obtained during the quarantine period, the introduced animals and
their offspring would be destroyed immediately and the facility sterilized.

In addition to the ICES code, signatories to the GATT and WTO are bound by the Agreement on the Application of Sanitary and Phytosanitary Measures (the “SPS Agreement”). The SPS Agreement applies to all sanitary and phytosanitary measures that may, directly or indirectly, affect international trade, including trade in live organisms. It permits sanitary and phytosanitary measures necessary to protect human, animal or plant life or health, but only if they are based on scientific principles. They cannot be maintained without sufficient scientific evidence, and must not arbitrarily or unjustifiably discriminate between members where identical or similar conditions prevail. In particular, it states that measures cannot be applied in a manner that would constitute a disguised restriction on international trade.

The risk of introducing exotic pathogens can be reduced significantly by using specific pathogen-free (SPF) stocks. However, in addition to possible deficiencies in SPF technology, other disease problems may develop from unknown pathogens in the imported populations. As we will see, this can be a particular problem with shrimp and other crustaceans.

Another approach with viral diseases is to develop resistant shrimp strains or to use shrimp species that are “specific pathogen resistant” (SPR), regardless of their pathogen status. However, “resistant” shrimp may sometimes carry the relevant virus as a persistent infection and be capable of introducing it in naïve populations. Even if a virus of the same name is already present in a native population, new strains may be introduced with SPR stocks and their prevalence might be maintained or increased. In addition, stress during farming can trigger increased viral replication in shrimp, resulting in disease outbreaks. There is also a danger that an exotic virus could mutate into a more pathogenic strain. This is particularly important for Taura syndrome virus (TSV) and yellow head virus (YHV), since they are RNA viruses known to mutate and evolve rapidly.

In the following paragraphs, I will try to outline why shrimp, other crustaceans and arthropods in general are much more dangerous than vertebrates for translocation. This is because they differ fundamentally from vertebrates in their mode of interaction with viruses. This difference results in the tendency for crustaceans to carry life-long, persistent infections without any gross or histological signs of infection. Past failure to appreciate this fact has resulted in 3 major shrimp epizootics that have collectively caused many billion dollars (US) economic loss since the beginning of large-scale shrimp cultivation in the early 1980’s. I will give a brief review of the nature of shrimp-virus interactions, a summary of the 3 epizootics and some suggestions of how to avoid similar future occurrences.

The Shrimp Response to Viral Pathogens

Little has been done on the mechanistic response of arthropods to viral pathogens. Interest has changed dramatically due to urgency in solving problems with massive losses in penaeid shrimp cultivation due to viral pathogens. Results of laboratory experiments in the past decade indicate that shrimp and other arthropods respond to viruses in a manner fundamentally different from vertebrates (Table 1) and are capable of a specific, adaptive response that cannot be explained by current knowledge and understanding of their cellular and humoral defenses. A hallmark of the arthropod response is specific tolerance to single and multiple viral infections without gross or histological signs of disease. The concept of viral accommodation was introduced in 1998 as a simple testable hypothesis to explain this tolerance. Key elements of the hypothesis were an unknown mechanism for specific host memory of pathogens and a role for this memory in dampening viral-triggered apoptosis.

Recent field and laboratory research results with shrimp and insects support the predictions of the viral accommodation hypothesis and suggest that memory is provided by the viral pathogens themselves in persistent infections that reduce the severity of disease. The well-known phenomenon of defective interfering viral particles (DIP) probably plays an important role in this process, but it cannot explain cross protection that has recently been described for

Table 1. General characteristics of shrimp and vertebrate responses to viral pathogens. The table contrasts dominant features and does not address “exceptions to the general rule”. The purpose is to point out broad features of difference that would have to be accounted for in any models proposed to explain the shrimp response to viral pathogens.

<table>
<thead>
<tr>
<th>Shrimp</th>
<th>Vertebrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>No response against pathogen</td>
<td>Response against pathogen</td>
</tr>
<tr>
<td>Survivors usually infected</td>
<td>Survivors usually not infected</td>
</tr>
<tr>
<td>– i.e. pathogen persists</td>
<td>– i.e., pathogen cleared</td>
</tr>
<tr>
<td>Infectious for others</td>
<td>Not infectious for others</td>
</tr>
<tr>
<td>Tolerance to viruses normal</td>
<td>Tolerance to viruses rare</td>
</tr>
<tr>
<td>No antibodies found in serum</td>
<td>Antibodies found in serum</td>
</tr>
<tr>
<td>Multiple active infections</td>
<td>Multiple active infections rare</td>
</tr>
<tr>
<td>Extremely heavy viral</td>
<td>Moderate viral production</td>
</tr>
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<td>production</td>
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heterologous viral infections in shrimp and insect cells. Homologous and heterologous reduction in disease severity as a result of persistent viral infections may be a key process that has evolved from host-viral interaction in the arthropod line.

Other recent publications have shown that prior exposure of shrimp to inactivated viral particles or envelope proteins may protect them from lethal viral challenge for a short period of time. By contrast, persistently infected shrimp appear to maintain protection as long as they remain infected. Since treatment with inactivated viruses or coat proteins runs out relatively quickly, it should not be called vaccination and compared to long-term protective, antibody-based, immunological memory, such as that gained by vaccination in vertebrates. There is no evidence that shrimp or other arthropods have a comparable defense system. Rather, the evidence in hand suggests that “memory” when it exists, is provided by continuous presence of the pathogen (i.e., “the virus is the memory”). This being the case, one would expect protection to be limited by the range of interfering particles produced. In other words, it would be expected to have specificity, as has earlier been proposed. However, heterologous protection remains unexplained.

Whatever the mechanism and whatever reason, it is indisputable that shrimp, other crustaceans and arthropods (including insect cell lines) can carry persistent viral infections without signs of disease. The following examples will show that failure to appreciate this fact has resulted in widespread shrimp epizootics.

**Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV)**

IHHNV was first discovered in blue shrimp *Penaeus stylirostris* and white shrimp *P. vannamei* (also called *Litopenaeus stylirostris* and *L. vannamei*) in the Americas in the early 1980s, where it was believed to have been introduced by importation of live experimental stocks of the black tiger shrimp *Penaeus monodon* from Asia. It is important to understand that IHHNV was unknown before this jump from *P. monodon*. It was unknown because it generally produces no adverse effects in *P. monodon*, including no gross or histological signs of disease. Often it can be detected only by using polymerase chain reaction (PCR) assays. Although IHHNV has been reported to occur in several species of wild and cultured penaeid shrimp throughout the world, it has been reported to cause acute epizootics and mass mortality only in *P. stylirostris*, especially in juveniles and sub-adults. By contrast, it does not cause mortality in *P. vannamei*, but rather reduced, irregular growth and cuticular deformities, gross signs collectively referred to as “runt-deformity syndrome” (RDS). In spite of no mortality, commercial losses from RDS can be high. *P. stylirostris* and *P. vannamei* that survive IHHNV epizootics may carry the virus for life and pass it on by vertical and horizontal transmission. The infected adult carriers show no signs of disease or mortalities. Vertically infected larvae and early postlarvae of *P stylirostris* do not become diseased, but massive mortalities may occur in juveniles at approximately 35 days or more. As stated previously, *P monodon* is relatively unaffected by IHHNV while *P. indicus* and *P. merguiensis* (also called *Fenneropenaeus indicus* and *F. merguiensis*) appear to non-susceptible.

This was the first example of a very costly epizootic caused by an unknown virus that jumped from one grossly healthy shrimp species to another. Unfortunately, the lesson went unheeded and subsequent epizootics by new viruses also occurred via grossly normal broodstock and fry.

**Taura Syndrome Virus (TSV)**

The Taura syndrome (TS) was first described as a shrimp disease in Ecuador in 1992. Both toxic and infectious aetiologies were considered. An infectious agent was subsequently described in 1995 and named Taura Syndrome virus or TSV. However, the authors of the original Taura syndrome report disputed that TSV was the cause of TS and recommend that TSV be called instead infectious cuticular epithelial necrosis virus (ICENV). The history of the dispute has been reviewed. Here, the virus will be referred to as TSV. TSV is a cytoplasmic, non-enveloped icosahedral virus, 32 nm in diameter. It has a buoyant density of 1.338 g/ml and its genome consists of a linear, positive-sense ssRNA of approximately 10.2 kb. It has recently been classified in the family *Dicistroviridae* together with cricket paralysis viruses. TSV was a serious cause of shrimp mortality for reared *P vannamei* in the Americas, where it spread principally through regional and international transfer of live postlarvae and broodstock. More recently, it has been reported from *P vannamei* reared in Taiwan after importation of live shrimp stocks from the Americas. It has also now been reported from Thailand. Although TSV infects a number of penaeid species, it has caused serious commercial losses only for juvenile to adult stages of *P vannamei*.

The disease caused by TSV has three distinct phases (acute, transition and chronic), although only the acute and transition phases show distinguishable gross signs. Gross signs for moribund *P. vannamei* in the acute phase include overall pale reddish coloration and distinctly red tail fans and pleopods (i.e., “red tail” disease), where focal necrosis of the cuticular epithelium can be seen with a 10x hand lens. These shrimp also
show gross signs of soft shells and empty guts and usually die during molting. The transition phase in TSV epizootics shows gross signs of random, multi-local, irregularly shaped melanized cuticular areas that mark resolving TSV lesions. Affected shrimp may or may not have soft cuticles and red-coloration and may behave and feed normally\textsuperscript{33, 34, 44}. If shrimp with these black lesions survive the next molt, the lesions disappear and they appear grossly normal, despite the continuing presence of the virus, especially in the lymphoid organ\textsuperscript{45, 46}. Thus, TSV survivors can grow through to adults as infected but grossly normal animals and can produce infected PL that also appear to be normal.

The cultivation of exotic Penaeus vannamei in Asia began in earnest in Taiwan in the late 1990’s. Success with imported SPF stocks from the Americas led to rapid adoption and a rapid rise in demand for PL. This resulted in careless importation of grossly normal but infected shrimp from the Americas and subsequent outbreaks of TSV\textsuperscript{40}. A similar scenario in Thailand in 2001-2002 led to the first TSV outbreaks in late 2002, probably originating from shrimp imported live for aquaculture from Taiwan and China\textsuperscript{42, 47}.

White Spot Syndrome Virus (WSSV)

Although WSSV initially caused serious shrimp production losses only in Asia\textsuperscript{48}, it must now be considered the single most serious shrimp pathogen worldwide. It was first reported from farmed Penaeus japonicus in Japan in 1993\textsuperscript{49-53} and called penaeid rod-shaped DNA virus (PRDV) or rod-shaped nuclear virus of P. japonicus (RV-PJ)\textsuperscript{49, 50, 52, 54, 55}. Similar rod-shaped viruses from elsewhere in Asia were called by various names\textsuperscript{48}, but Lightner\textsuperscript{44} grouped them in a single white spot syndrome virus (WSSV) complex. WSSV is now included in a new virus family called Nimaviridae and a new genus Whisposviridae\textsuperscript{38, 36}. Captured broodstock and fry used to stock rearing ponds are known to carry WSSV, as are numerous other crustaceans and perhaps even aquatic insect larvae, but massive mortality usually occurs with juvenile shrimp in rearing ponds, probably precipitated by environmental factors\textsuperscript{18}.

In Thailand, outbreaks of WSSV in shrimp culture ponds initially occurred in 1994\textsuperscript{57, 58} and caused a peak estimated lost production of 70,000 metric tons in 1996. The total, cumulative lost production for all Asian countries since 1993 must now amount to more than 1 million tons, while the world loss, including those from the USA, Central America and South America must be very much higher. The same or very closely related variants appear to be the cause of all these losses\textsuperscript{56-60}.

The WSSV outbreaks in Japan were the first widely reported, but they actually followed Chinese outbreaks and apparently resulted from the import of grossly normal, living post larvae from China directly to aquaculture facilities in Japan\textsuperscript{51}. No one knows how the virus spread throughout Asia after that, but the common practice of moving grossly normal broodstock and PL freely amongst countries was probably the most rapid and effective means of spread. Almost certainly WSSV was spread from Thailand to Malaysia and India in this manner. In addition, WSSV was not reported from the Philippines until 2000\textsuperscript{61}, probably because of an effective Philippine government ban on importation of broodstock and post larvae. Anecdotal evidence suggests that the Philippine outbreaks in the late 1990’s originated from illegal import of post larvae from China. As in Asia, a good part of WSSV spread in the Americas probably resulted from international transport of live shrimp for aquaculture\textsuperscript{62}.

It is curious that WSSV had not been reported from China prior to the catastrophic disease outbreaks in 1993, in spite of the fact that shrimp aquaculture had been practiced there for many years. The nature of the outbreaks was reminiscent of the initial IHHNV outbreaks for P. stylirostris in the Americas, suggesting that they may have originated from importation of a distant, exotic aquaculture species carrying a previously unknown pathogen. Whatever the original source, it is clear that the majority of the subsequent geographical spread was via grossly normal broodstock and PL.

Lessons Unlearned

Experience with these 3 large-scale epizootics in shrimp has shown that spread of disease usually resulted from translocation of grossly normal broodstock or PL. At the initial phase of all 3 cases, the causal agent was unknown and appropriate diagnostic tools were developed only after the fact. With IHHNV, the original source of the virus has been identified, but for TSV and WSSV, it remains to be determined. However, good guesses might be a jump from a carelessly imported exotic crustacean to P. monodon for the case of WSSV in China/Taiwan and a jump of a native crustacean pathogen to exotic P. vannamei in the case of TSV in the Americas. In any case, the take-home lesson is that grossly normal shrimp are capable of carrying one or more unknown viral pathogens without gross or histological signs of disease. Despite this knowledge and the availability of appropriate diagnostic tools, TSV was transferred to both China/Taiwan and Thailand. Obviously, forewarning and existing mechanisms to prevent the transfers failed.

The consequence of TSV transfer to Thailand has been the development of Thai genetic variants that differ from those in the USA and China\textsuperscript{63} and have been found in native species, in addition to the exotic P. vannamei. Since virulence differs amongst American genetic variants\textsuperscript{63, 64, 43}, we might expect the same to be
true of those developing in Thailand. The long-term consequences for native species is unknown. In addition to this, a new problem called monodon slow growth syndrome (MSGS) began to occur widely in Thailand in 2002. The rapid, country-wide occurrence of the problem, its tendency to be related to PL batch and the lack of association with known pathogens suggested the possible occurrence of a new infectious agent (MSGA). No conclusions can yet be made regarding this speculation, but concurrence of the problem with large-scale import and cultivation of *P. vannamei* opens the possibility for yet another new, cryptically introduced virus.

**Recommendations**

Given the propensity of shrimp, crustaceans and other arthropods to carry cryptic viral infections, it is reasonable to suggest that ICES guidelines be modified and closely followed to guard against the possibility of introducing new viral pathogens. It is not sufficient to certify the exotic animals as free (SPF) for a list of known pathogens with available diagnostic tests. There is additional danger from previously unknown viruses or variants of known viruses for which no assay methods exist.

Given the experience with IHHNV in *P. monodon*, it would seem prudent to add to the ICES guidelines a special requirement for shrimp and other crustaceans. This would be the requirement that native species of shrimp and other economically important crustaceans be included as co-habitants in the quarantine phase of the importation process. This would guard against the unintentional transfer of any well-tolerated, unknown pathogen from the exotic host to local species that might be more vulnerable and more seriously affected. Doing this would have avoided the release of IHHNV in the Americas, WSSV in Japan and TSV in Taiwan. However, to make the guidelines work, standard protocols for inspection, disease diagnosis, and certification of shipments of live marine animals would be required, and the infrastructure for this in Asia is not always in place. Most effective would be increased awareness of the potential threats among aquaculture practitioners themselves and their cooperation in guarding against it.

Outright import bans on all crustaceans might be an effective exclusion measure, but it would unreasonably deprive aquaculturists of potentially good alternative species for cultivation. A better approach would be promotion of joint-venture operations with developers of domesticated or genetically improved stocks to establish local breeding centers via the modified ICES protocol. Once safely established, the costly process of continuous importation and screening of stocks could be avoided.

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