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Infectious Hypodermal and Haematopoietic Necrosis Virus (IHHNV)

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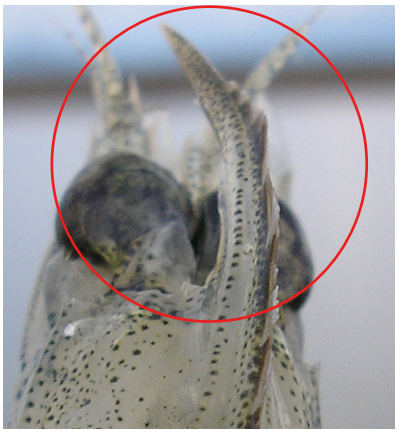
Shrimp get sick too. IHHNV infects wild and farmed shrimp, and has been detected by PCR in non-penaeid shrimp, crabs and fishes. This virus is highly contagious and causes high mortality in *P. stylirostris* where the disease is known as "Infectious hypodermal and haematopoietic necrosis" (IHHN). There is no evidence of mortality in *P. vannamei,* where virus effects include slow growth rate and body deformities; in *P. vannamei* disease is known as "Runt deformity syndrome" (RDS). In *P. monodon*, IHHNV infection may be present in asymptomatic individuals or clinical signs may be observed as shell deformities related to short rostrum and protruded abdominal segment joints.

Causative agent of Infectious Hypodermal and Haematopoietic Necrosis Disease and Runt Deformity Syndrome (RDS). This infectious disease or syndrome is caused by a DNA virus from the genus Penstyldensovirus, Family Parvoviridae. Virus multiplication (replication) occurs in the cell nucleus. IHHNV is the smallest known virus of penaeid shrimp with a virion of 20–22 nm, non-enveloped and shape of icosahedron. Its genome (genetic information) is composed by a linear single-stranded DNA. The Shrimp **Multi**Path PCR test will help to confirm IHHNV infections, as well as provide information to producers about presence and/or absence of this virus and other shrimp pathogens frequent in culture systems, in a precise, reliable and quantitative way (number of pathogens per sample).

Three different IHHNV genotypes have been reported: Type 1 (Americas and East Asia), Type 2 (South-East Asia) and Type 3 (Australia, China and Taiwan). These IHHNV lineages may infect *P. vannamei, P. stylirostris* and *P. monodon*. It seems that IHHNV can insert part of its DNA into the genome (DNA) of penaeid shrimp; it's known as an endogenous viral element (EVE) and has been described in shrimp of East Africa, India and Australia, the same as in Western Indo-Pacific including Madagascar, Mauritius and Tanzania. However, shrimp DNA containing the IHHNV-homologous sequences are not infectious to susceptible penaeid species like *P. vannamei* or *P. monodon,* but will cause false positive PCR data if assays are not designed correctly to differentiate between inserted IHHNV DNA and infectious viral particles.

Whilst the virus has no impact on human health or food safety, and any affected shrimp are safe for human consumption, it does have a significantly detrimental effect for shrimp farmers. IHHNV can infect all penaeid shrimp life stages, including eggs, larvae, postlarvae, juveniles and adults. IHHNV-infected females with high virus loads usually produce eggs that fail to develop and hatch. Nauplii obtained from infected broodstock, present high prevalence of IHHNV infection. The impacts of IHHNV on production outputs include reduced survival, reduced growth, a less uniform size at harvest and overall lower size class. Deformities seen as part of RDS also reduce harvest value.
Field observations and laboratory experiments have shown that IHHNV infection may delay mortality in populations infected with White Spot Syndrome Virus (WSSV) due to possible viral competition.

Clinical signs. Evident cuticular deformities like deformed rostrum bent to either side can be observed in *P. stylirostris, P. monodon* or *P. vannamei* with RDS, which may be considered as pathognomonic for IHHNV infection. Nevertheless, this clinical finding is not always present in infected populations. See image right for an example.



As penaeid shrimp can harbour IHHNV infection without evidence of obvious clinical signs, it is recommended to run Shrimp **Multi**Path PCR to identify infected broodstock for removal from spawning populations, identification of IHHNV positive postlarvae prior to stocking for exclusion where possible, and monitoring of IHHNV during commercial grow-out in order to better manage pond inputs and management. Shrimp **Multi**Path is designed to only detect pathogenic IHHNV, meaning false positive detections from non-pathogenic IHHNV EVE in the Shrimp genome does not occur on this platform.

Clinical signs in *P. stylirostris*. IHHNV infection in *P. stylirostris* usually produces high mortality in juvenile stages. In spite of the infection, vertically transmitted virus in larvae does not result in clinical signs and disease whilst shrimp are young. However, juveniles with one month after stocking present gross signs of the disease and mass mortalities. Horizontal infection in juveniles has an incubation period and disease severity that are size and age dependent. After vertical transmission, IHHNV infection affect normal development of early stages (eggs, larvae and postlarvae). Clinical signs in *P. stylirostris, P. vannamei* and *P. monodon,* can be observed in young juveniles, which suggests a virus incubation period of just 3 to 5 weeks.

Gross signs in *P. stylirostris* juveniles infected with IHHNV include low feed consumption, behaviour and appearance changes, slow rise of shrimp to the water surface, motionless, roll-over and slowly sink (ventral side up) to the pond bottom. During this behaviour, shrimp can be cannibalised by healthy shrimp. IHHNV infected *P. stylirostris* also may present white or buff-coloured spots (different to WSSV infection in appearance and location of the spots) in cuticular epidermis, especially under tergal plates of the abdomen with a mottled appearance. Moribund juveniles of *P. monodon* and *P. stylirostris* look bluish with opaque abdomen.

Clinical signs in *P. vannamei* suffer a chronic infection with IHHNV known as "Runt deformity syndrome" (RDS). Infection with IHHNV can occur during larval or postlarval stages, which may determine severity and prevalence of the disease. RDS has been observed in farmed populations of *P. monodon* and *P. stylirostris* juvenile as well. Sick shrimp with IHHNV display a rostrum bent at 45° - 90° right or left. Also deformed abdominal segments, cuticular roughness, wrinkled antenna, bubble-like heads, and other cuticular deformities. Juvenile populations with RDS present irregular growth, wide size distribution and a weight coefficient of variation (CV) up to 30% (normal CV in healthy populations are 10–20%).

Clinical signs in *P. monodon*. Although this species is usually asymptomatic for IHHNV infection, some acute *P. monodon* infections with this virus may produce shell deformities that include shortened rostrums and protruded abdominal segment joints easily observed in sick organisms.

Early detection using Shrimp MultiPath PCR testing before clinical signs are apparent, can give farmers early notice to mitigate disease spread and maximise production outputs. It's important to establish early IHHNV disease mitigation strategies. These may include viral exclusion programs which involves the use of PCR tools in order to confirm when broodstock or postlarvae are "positive" to IHHNV. These data can be used to inform rejection of these shrimp or batches from production. If IHHNV is detected in grow-out ponds, disease expression risk may be reduced by avoiding physico-chemical parameter abrupt changes and keeping environmental conditions as stable as possible.

Target life-history stages for accurate early detection include larvae or postlarvae stages, juveniles, subadults and adults. When juveniles or subadults are obtained from ponds suspicious of IHHNV infection, smaller shrimp must be sampled to be subjected to PCR tests. Nevertheless, due to vertical transmission of IHHNV, it's also possible to find this virus in eggs. In grow-out ponds where IHHNV infection is suspected, weight coefficient of variation (CV) must be over 30%. This is established by weighing >100 randomly (cast-net) captured shrimp. In *P. monodon*, it is suggested PL8 stage as the more accurate size for early IHHNV detection by PCR.

Target organs for sensitive PCR detection are haemolymph, pleopods and gills. Samples of haemolymph, pleopods or gill filaments can be taken non-lethally if a farmer is testing valuable broodstock. Whole PL8-15 can also be sampled for testing, or PL heads.

Sampling and preservation of tissues for PCR tests should be done in labelled vials or tubes that seal and fixative should be 70-95% laboratory grade ethanol; freezing samples will also preserve virus DNA. Tissue size can be 2-5 mm² in size (50 mg approx.). Sample equipment must be sterilied between sample tubes.

Sampling numbers and Health Management Plans should be established with your health expert who will take into account factors such as nauplii/postlarvae source, climate, farm size and location, company structure, market channels for sale of product etc. There is also the option to pool samples for IHHNV testing to maximise value for money with PCR testing.

Longer term solutions to IHHNV prevalence include breeding for tolerance and resistance, PCR-based exclusion programmes, and the use of developing "RNA interference antiviral" techniques (RNAi). Early pathogen detection and risk mitigation through the use of Shrimp **Multi**Path is also a foundational approach to solving IHHNV pond consequences.

Contact Genics at info@genics.com if you would like to discuss these options for your operation or visit www.genics.com for further details. Watch the instructional video on Shrimp **Multi**Path target organ dissection below.

Questions?

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IHHNV

Did you know...

Shrimp rarely just harbour only one pathogen and farmers often don't know which ones they are. This is a huge economic risk for farmers. **Genics has solved this problem with** Shrimp **Multi**Path**.** It stacks up as the ultimate early warning system for farmers, detecting 13 pathogens in a single automated test that is unparalleled in today's industry for its sensitivity and accuracy.