Yellowhead Disease

**Agent Description**

Yellowhead Disease is considered to be infection with Yellowhead Virus (YHV). YHV (genotype 1) is one of six known genotypes in the yellowhead complex of viruses and is the only known agent of yellowhead disease. YHV and other genotypes have been classified as a single species in the genus *Okavirus*, family *Roniviridae*, order *Nidovirales*. Virus particles appear rod shaped with a helical nucleocapsid showing parallel cross-striations, prominent surface projections and measure c. 40 x 200nm.

YHV is a positive sense ssRNA virus and replicates within the cytoplasm of infected host cells. Gill-associated virus (GAV) is designated as genotype 2 of the Yellowhead complex and occurs with four other known genotypes (genotype 3-6) in healthy *Penaeus monodon* in East Africa, Asia and Australia. Genotypes 2-6 are rarely associated with disease in these regions. Sequencing of ORF1a and ORF1b has aided the discrimination of YHV (genotype 1) from GAV and the other viral genotypes in the complex.

**Stability**

YHV remains viable in aerated seawater for up to 72 hours. Heating to 60°C for 15 minutes and exposure to 30 ppm chlorine can inactivate YHV.

**Replication**

Clinical signs of YHD occur in *P.monodon* within 7-10 days of exposure.

**Transmission**

YHV has been transmitted horizontally by injection and ingestion of infected material and by immersion in membrane-filtered tissue extracts or by co-habitation with infected shrimp. GAV has been demonstrated to transmit vertically, probably by surface contamination or infection of tissue surrounding the eggs.

**Prevalence**

Prevalence of viruses within the complex can be high (50-100%) in farmed and wild populations of *P. monodon* in Australia, East Africa and Asia but the prevalence of YHV (genotype 1) in healthy wild or farmed *P.monodon* is generally (<1%), increasing to very high (up to 100%) during Yellowhead disease outbreaks in ponds.

**Geographical distribution**

YHV - People’s Republic of China, India, Indonesia, Malaysia, the Philippines, Sri Lanka, Taipei China, Thailand and Vietnam. GAV and other genotypes – Australia, India, Indonesia, Malaysia, Mozambique, the Philippines, Taipei China, Thailand and Vietnam.

**Vectors**

No known vectors of YHV though co-existing pond invertebrates may be expected to act as mechanical vectors.

**Mortality**

YHV can induce up to 100% mortality in infected *P.monodon* ponds within 3 days of the first appearance of clinical signs. YHV infected shrimp demonstrate frequent co-infections with bacterial pathogens, these secondary opportunistic pathogens may play a role in the eventual mortality of YHV infected shrimp. Despite being considered as a less pathogenic genotype within the complex, GAV has been associated with mortalities up to 80% in *P.monodon* ponds in Australia.
Control and Prevention
No effective vaccines for viruses within the Yellowhead complex are available. Use of specific pathogen free (SPF) or PCR-negative seed stocks and use of biosecure water and culture systems is recommended.

Gross Pathology

Penaeid shrimp
Moribund shrimp may exhibit a bleached overall appearance and a yellowish discoloration of the cephalothorax caused by the underlying yellow hepatopancreas, which may be exceptionally soft when compared with the brown hepatopancreas of normal shrimp. YHV can infect cultured shrimp from late postlarval stages onwards, but mass mortality usually occurs in early to late juvenile stages. High feeding activity may be followed by an abrupt cessation of feeding 2-4 days following the appearance of gross clinical signs of disease and mortality. Moribund shrimp may congregate at pond edges near the surface. In many cases, total crop loss can occur within a few days of the first appearance of shrimp showing gross signs of the disease. External signs are not pathogen specific. Therefore gross observations should not substitute more specific methods for diagnosis.

Similarly, gross signs of GAV disease include swimming near the surface and at the pond edges, cessation of feeding, a reddening of body and appendages, and pink to yellow colouration of the gills. However, these signs occur commonly in diseased shrimp are not considered a reliable method for diagnosis of GAV disease.

Crabs, crayfish, freshwater prawns, spiny lobsters and clawed lobsters
Unknown

Clinical Pathology

Histology
Systemic necrosis of ectodermal and mesodermal tissues with prominent nuclear pyknosis and karyorrhexis. Cells contain moderate to large numbers of deeply basophilic, evenly stained, spherical, cytoplasmic inclusions approximately 2µm in diameter or smaller. The lymphoid organ, haemocytes, haemopoietic tissue, gill, heart, cuticular epithelium, midgut and connective tissues are the primary target tissues and organs for YHV infection. Cellular changes in early infections may include nuclear hypertrophy, chromatin diminution and margination, and lateral displacement of the nucleolus. Loss of tissue structure within lymphoid organ, stromal matrix cells that comprise tubules become infected leading to loss of tubular structure, tubules appear degenerate. Lymphoid organ spheroids (LOS) develop during infection, ectopic spheroids may lodge in constricted areas of the haemocoel (heart, gills, subcuticular connective tissues etc). Necrosis of the lymphoid organ in YHD infections can be used to distinguish YHD from acute Taura Syndrome (TS) in penaeid shrimp. Note: the lack of a lymphoid organ in several other host groups within the Decapoda prevent this being used as a target for assessing clinical pathology in these hosts. Representative images of YHV histopathology are provided in Annex 1.

Transmission Electron Microscopy (TEM)
Nucleocapsid precursors and complete enveloped virions can be observed in YHV infected cells. Nucleocapsid precursors are long filaments approximately 15nm in diameter and of variable length (80-450nm) that occur in the cytoplasm, sometimes densely packed into paracrystalline arrays. Assembled Virions are rod-shaped and
enveloped, measuring 40-60nm x 150-200nm with rounded ends and prominent surface projections (8-11nm). Virions can be found in the cytoplasm of infected cells, in association with intracellular vesicles and can be seen budding at the cytoplasmic membrane and in interstitial spaces. GAV virions and nucleocapsids are indistinguishable from YHV by TEM. Representative images of YHV TEM are provided in Annex 1.

Susceptible Species

A comprehensive assessment of host susceptibility to viruses in the Yellowhead complex has recently been completed by EFSA (EFSA 2008). The assessment takes into account the key susceptibility criteria of pathogen replication, bioassay, characteristic pathology and anatomical location of pathogen and has critically assessed the available literature on host-pathogen interaction with respect to Yellowhead complex viruses. Gulf brown shrimp (Penaeus aztecus), Gulf pink shrimp (P. duorarum), Kuruma prawn (P. japonicus), black tiger shrimp (P. monodon), Gulf white shrimp (P. setiferus), Pacific blue shrimp (P. stylirostris), and Pacific white shrimp (P. vannamei) are currently listed as species susceptible to Yellowhead virus complex genotypes in EC Directive 2006/88. However, the EFSA assessment identified scientific literature to confirm susceptibility in 18 potential host species. Using the EFSA principles of susceptibility, scientific data are available to support susceptibility of P. monodon, P. merguiensis, P. vannamei, P. setiferus, P. aztecus, P. duorarum, Metapenaeus brevicornis, M. affinis and Palaemon styliferus. Additionally, there are scientific data suggesting susceptibility of P. esculentus, P. japonicus, P. stylirostris, Metapenaeus ensis, and M. bennettsae though some uncertainty exists regarding the identity of the specific pathogen within the complex being assessed. Information on P. esculentus, M. ensis, M. bennettsae, and Macrobrachium lanchesteri was considered insufficient to scientifically assess susceptibility with regards to the EFSA criteria. Scientific data suggesting susceptibility of M. lanchesteri, M. sintangense, and Palaemon serrifer are essentially experimental and invasive. With the exception of P. japonicus, no temperate water species have been specifically tested for susceptibility to viruses in the Yellowhead complex.

OIE Recommended Techniques for Surveillance and Confirmation

Use the methods listed in the table below for screening and confirmation

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Surveillance Techniques

RT-Polymerase Chain Reaction (RT-PCR)

The OIE recommends three RT-PCR protocols. The first is a one-step RT-PCR based on that of Wongteerasupaya *et al.* (1997) used for confirmation of YHV in shrimp from suspected Yellowhead disease outbreaks. This test will not detect GAV or other genotypes in the complex. The second is a more sensitive multiplex nested
RT-PCR protocol adapted from Cowley *et al.* (2004) that can be used for differential detection of YHV and GAV in disease outbreaks or for screening. The third (unpublished) protocol is based on a sensitive multiplex RT-nested PCR procedure by Wijegoonawardane, Cowley and Walker that can be used for screening healthy shrimp for viruses in the YHD complex and detects all six currently known genotypes. The test does not however distinguish between the genotypes though this can be achieved by nucleotide sequence analysis of the RT-PCR product. Details of these test protocols are available in the most recent OIE Manual of Diagnostic Tests for Aquatic Animals).

**Confirmatory Tests**

**Transmission Electron Microscopy (TEM)**
Anaesthetise the crustacean by immersing in ice until immobilised. Quickly dissect small blocks (2 mm\(^3\)) of gill and lymphoid organ tissue (in penaeid shrimp) and fix for in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for at least 2 h at room temperature. Rinse fixed tissue samples in 0.1 M sodium cacodylate buffer (pH 7.4) before post-fixation for 1 h in 1 % osmium tetroxide in 0.1 M sodium cacodylate buffer. Rinse samples in 0.1 M sodium cacodylate buffer before dehydrating through a graded acetone series. Embed samples in an epoxy resin and polymerise according to manufacturers guidelines. Semi-thin (1-2 µm) sections are stained with Toluidine Blue for viewing with a light microscope to identify the suitable target areas. Ultra thin sections (70-90 nm) of target areas are mounted on uncoated copper grids and stained with 2% aqueous uranyl acetate and Reynolds’ lead citrate (Reynolds 1963).

**In situ hybridisation (ISH)**
The suggested protocol is that developed by Tang & Lightner (1999), this method is suitable for detection of YHV and GAV.

**PCR**
As for surveillance

**Sequencing**
Two-step PCR and sequencing are the prescribed methods for declaring freedom. Where a two-step PCR positive result cannot be confirmed by sequencing this should count as a negative result. Sequencing of the ORF1a and ORF1b regions can be utilised to discriminate YHV (genotype 1) from other viruses in this complex.

**EU-legislation**

Yellowhead Disease is listed in the new EU Directive 2006/88/EC as an exotic pathogen.

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References


Annex 1 Histopathology and TEM of YHD caused by YHV (genotype 1)

Fig. 2 A and B. Histopathology of YHV infection in heart (A) and subcuticular epidermal cells (B) of *P. vannamei*
Fig. 1 A and B. Histopathology of YHV infection in lymphoid organ of *P. vannamei*
Fig. 3. Histopathology of YHV infection in gill (A); and TEM of YHV viral precursors and particles in gill (B, C, D), of *P. vannamei*