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 POST LARVAE/HEPATOPANCREATIC PARVOVIRUS

JURAIAT PIROMJAI : THE APPLICATION OF GENE PROBES TO
 DETECT HPV OF PENAEUS MONODON BY *IN SITU* HYBRIDIZATION,
 DOT BLOT HYBRIDIZATION AND PCR AMPLIFICATION. THESIS
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Hepatopancreatic parvo virus (HPV) infects the hepatopaereas in panaeid shrimp. It may cause stunting of growth or death to shrimp and can thus cause reduced harvests for shrimp farmers. This work was based on the fact that commercial PCR primers developed for HPV from *Penaeus chinensis* (HPVchin) produced a unique 732 bp DNA fragment for HPV from *P. monodon* (HPVmon). Analysis of the nucleotide sequence of this unique DNA fragment showed that it was 696 bp in length and contained 47% GC content. A sequence alignment of the 696 bp HPVmon DNA fragment gave 84% homology with the HPVmon genome, while alignment of a portion of it gave 76.7% homology with a 350 bp DNA fragment of HPVchin.

Specific primers for HPVmon (H441F and H441R) were designed from this 696 bp DNA fragment. These primers gave a 441 bp product with as little as 1 fg template consisting of purified HPVmon DNA while no product was obtained from any other DNA templates derived from other viral pathogens or from *P. monodon*. In direct PCR assays using H441 primer set to detect HPVmon DNA added to shrimp faecal and post larval samples preserved in lysis buffer, no PCR amplicons were obtained. However, if DNA was first extracted from post larvae homogenized in lysis buffer, added (spiked) HPVmon DNA could be detected by this PCR reaction at as little as 1 pg. The detection limit for HPVmon DNA added to faecal and post larval samples preserved in 0.025%NaOH and 0.0125%SDS was 0.1 pg.

A DIG-labeled PH441 probe was prepared from the 441 bp HPVmon amplicon using a PCR labeling kit and this probe was used for HPV detection by dot blot hybridization and *in situ* hybridization.

By dot blot hybridization, this probe gave no specific signal when using templates derived from shrimp culture water, shrimp food, shrimp faeces (HPV-free), *V. parahaemolyticus*, *P. monodon* and other shrimp viruses (both DNA and RNA). The probe did give a visible signal with purified HPV DNA at the lowest concentration of 0.01 pg. When used for detection of HPVmon DNA in spiked faeces and post larval samples, a strong intense signal was visible down to 1 pg and 0.1 pg spiked HPVmon DNA, respectively.

Based on *in situ* hybridization, PH441 probe gave a blue-black staining reaction with HPV infected nuclei of hepatopancreatic cells from *P. monodon*, whereas no signals were found in slides prepared from normal shrimp or shrimp infected with other viruses.