

野田病毒基因型分析與在細胞株 E-11 之感染特性

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摘要

病毒神經性壞死症(Viral nervous necrosis disease; VNND)是造成養殖漁業經濟損失的重要疾病之一，並在幼魚時期造成高死亡率。病原為神經壞死病毒(Nervous necrosis virus ; NNV)，屬於 *Nodaviridae* 科、*betanodavirus* 屬。

在台灣第一件神經壞死病毒病例於 1994 年被證實後，每年仍有疫情發生，2010 年我們擴大收集來自嘉義、屏東地區的隻魚隻樣本共 738 件，RT-PCR 檢測中 NNV 陽性率為 12.6% (93/738)，並在首次檢體中於日本鰻、蓋斑鬥魚、烏魚、台拉燕魚、川紋笛鯛、四絲馬鮫、吳郭魚之魚種發現陽性案例出現，顯示 NNV 能廣泛地感染淡水魚及海水魚宿主。

我們以細胞 E-11 作為分離 NNV 之細胞株，CPE (cytopathic effect) 於感染後第三至五天後逐漸顯現，培養最適溫度為 26.5 °C，2010 年陽性案件病毒分離率為 34.4 % (32/93)。挑選來自 91~99 年間七株分離株，連續測定各代數(P2-P7 or P8)的病毒力價在 $10^{5.3}$ TCID₅₀/ml~ $10^{8.3}$ TCID₅₀/ml 之間。將 91、93、94、98 與 99 年所分離 NNV 病毒株與台灣其他已發表分離株(共 15 株)比對 RNA2 之鞘蛋白(coat protein)核苷酸(830 bp)與胺基酸序列，所有分離株皆屬於 RGNNV 基因型，相似度為 97.7 ~ 99 %。另外韓國、泰國、新加坡及中國發表之魚類 NNV 分離株也屬於同一基因型，顯示 RGNNV 是亞洲主要 NNV 流行病毒株，其分布具有地區流行性。

現今已知 NNV 可藉由垂直與水平感染傳播，防治策略除了篩選陰性魚隻、臭氧消毒外，數種疫苗形式也正在研發，包括死毒疫苗、DNA 疫苗、重組蛋白疫苗...等。然而在魚苗階段，魚隻大小(體長<5 cm)並不適合以注射方式免疫，遂操作較簡易、減少緊迫的施行方式會是研發疫苗時需考量的。

Genetic analysis and characteristics of betanodaviruses in cell line E-11.

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Abstract

Virial nervous necrosis disease (VNND) is one of important diseases that caused mass economical loss in aquaculture industry, and the highest mortality occurred at larval stage. Nervous necrosis viruses (NNV) are the pathogen of VNN classified as genus *betanodavirus* in the family *Nodaviridae*.

In Taiwan, since the first VNN case was identified in 1994, VNN disease has been continuously reported in cultured fish. We collected samples widely from Chiayi and Pingtung County in 2010, and 93 samples out of 738 were detected RT-PCR positive (12.6 %) for nodavirus. This is the first description of NNV infection case in Japanese eel (*Anguilla japonica*), Paradise Fish (*Macropodus opercularis*), Sea mullet (*Mugil cephalus*), Orbicular batfish (*platax orbicularis*), Red emperor (*Lutjanus sebae*), East Asian fourfinger threadfin (*Eleutheronema rhadinum*), Tilapia (*Oreochromis spp*), it shows that betanodavirus occurs in large population of fresh water and marine fish.

We use E-11 cell line for virus isolation, CPE (cytopathic effect) began at 3~5 day post-infection. Optimal virus reproducible temperature was 26.5 °C, the isolate rate was 34.4% (32/93) in RT-PCR positive samples. We picked 7 strains isolated from 2002 to 2010, virus titer at serial passages (P2-P7 or P8) was between $10^{5.3}$ TCID₅₀/ml ~ $10^{8.3}$ TCID₅₀/ ml. A RT-PCR product (830 bp, partial RNA2 coat protein gene) was amplified and sequenced from 15 strains isolated in 2002, 2004, 2005, 2009, 2010 and other Taiwanese published strains. The nucleotide and deduced amino acid sequences of all isolates were 97.7 ~ 99 % homologous with genotype red- spotted grouper nervous necrosis virus (RGNNV). Recent studies reported that NNV isolates from Korea, Thailand, Singapore and China also belong to RGNNV, indicated RGNNV was the major virus in Asia and geographic epidemic.

Vertical and horizontal transmissions have been demonstrated in NNV. In addition to broodstock screening and ozone sterilization control strategies, several types of vaccines have developed, including inactivated vaccine, DNA vaccine, recombinant protein vaccine, *etc.* However, the size of fry stage (length < 5 cm) is too small to be immunized by injection; then, an easy treating and less stress method need to be considered in vaccine development.