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Detection of white spot syndrome virus in filtered shrimp-farm water fractions and experimental evaluation of its infectivity in *Penaeus* (*Litopenaeus*) *vannamei*

Héctor M. Esparza-Leal [a,b,1], César M. Escobedo-Bonilla [c,1,2], Ramón Casillas-Hernández [b], Píndaro Álvarez-Ruíz [a], Guillermo Portillo-Clark [d], Roberto C. Valerio-García [c], Jorge Hernández-López [c], Jesús Méndez-Lozano [a], Norberto Vibanco-Pérez [e], Francisco J. Magallón-Barajas [d,*]

[a] *Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional-IPN, Unidad Sinaloa, Boulevard Juan de Dios Bátiz Paredes #250, Guasave, Sinaloa 81101, Mexico*
[b] *Programa de Doctorado en Ciencias en Biotecnología. Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Cd. Obregón, Sonora 85000, Mexico*
[c] *Centro de Investigaciones Biológicas del Noroeste, Unidad Sonora, Centenario Norte 53, Col. Prados del Centenario, Hermosillo, Sonora 83260, Mexico*
[d] *Centro de Investigaciones Biológicas del Noroeste, Mar Bermejo 195, Col. Playa Palo de Santa Rita, La Paz, Baja California Sur 23090, Mexico*
[e] *Universidad Autónoma de Nayarit, Edificio CEMIC 05, Ciudad de la Cultura Amado Nervo, Tepic, Nayarit 63190, Mexico*

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ABSTRACT

White spot syndrome virus (WSSV) may spread through water to neighbor ponds or farms. Routine water exchange and wastewater released during white spot disease (WSD)-emergency harvests may preserve WSSV in shrimp farming areas. To test this hypothesis, on-site experiments were performed in a WSSV-affected farm in Guasave, Sinaloa, Mexico. Plankton and shrimp hemolymph were collected from 12 ponds during a WSD outbreak. PCR analyses showed that 72% of the hemolymph pools (26 out of 36) were WSSV-positive. In contrast, only 14% (4 of 28) plankton samples (filtered through 10 and 0.45 µm) from three ponds (2, 7 and 10) were WSSV-positive. Plankton from pond 9 was WSSV-negative, but 14 days later, shrimp began to die. At this point, a differential filtration experiment was performed in pond 9. WSSV-positive samples were only found in three fractions [particulate fraction (PF) 1 µm and liquid fractions (LF) < 100 and < 40 µm]. Both LFs and PFs were used for *in situ* infectivity assays by water-borne routes in WSSV-negative whiteleg shrimp *Penaeus* (*Litopenaeus*) *vannamei*. Some shrimp exposed to different PFs and LFs (100 µm to > 0.65 µm) became WSSV-positive. Results indicate that water fractions between 100 and 0.65 µm induced WSSV infection to shrimp. Results showed that pond water and/or particulate fractions are vehicles for WSSV dispersion via virus suspended in water, attached to microalgae, or carried by zooplankton.

1. Introduction

White spot syndrome virus (WSSV) is a pathogen that has devastated the shrimp farming industry in several countries (Lightner et al., 1998; Jiang et al., 2006); currently it is the most serious shrimp viral pathogen in the world (Soto and Lotz, 2001; Flegel, 2006; Sánchez-Martínez et al., 2007). In Mexico, WSSV was first reported in 1999 and soon caused severe losses, first to the culturing of *Penaeus* (also called *Litopenaeus*) *stilyrostris* and later to *P. vannamei* (Galavíz-Silva et al., 2004; Peinado-Guevara and López-Meyer, 2006).

WSSV has a bacilliform shape and is a non-occluded, enveloped virus (Chou et al., 1995; Wongteerasupaya et al., 1995). Intact enveloped virions range from 210–380 nm in length and 70–167 nm in maximum width (Flegel and Alday-Sanz, 1998; Park et al., 1998; Rajendran et al., 1999; Escobedo-Bonilla et al., 2008). Under culturable conditions, this virus may cause up to 100% cumulative mortality in 2–10 days after the onset of symptoms (Lightner, 1996; Xu et al., 2006).

At present, no treatments are available to control the disease and mortality. The only alternative to reduce the risk of WSSV entry into commercial shrimp production facilities is the implementation of biosecurity or exclusion measures, such as filtration and disinfection (Clifford, 1999; Lightner, 2005). Water probably is a major pathway for WSSV entry into an aquaculture facility (Lotz and Lightner, 1999; Cohen et al., 2005; Corsin et al., 2005). Currently in shrimp farming, pond wastewater is routinely discharged into the adjacent environment (coastal lagoons or estuaries), where other crustacean species dwell. Many crustaceans are potentially susceptible to WSSV infection (see Escobedo-Bonilla et al., 2008). Heavy water exchange is normally done even in ponds affected by WSSV outbreaks. These practices probably increase the risk of WSSV transmission to neighboring shrimp farms.

Continuous and strict monitoring of the various components of shrimp farming is required to reduce the spread of WSSV within a region and to avoid introduction of the pathogen into a new area. These components include brooders (Hossain et al., 2004), fry, live

* Corresponding author. Tel.: +52 612 123 8484; fax: +52 612 125-3625.
E-mail address: fmagallon04@cibnor.mx (F.J. Magallón-Barajas).
[1] Both authors contributed equally to this paper.
[2] Present address: CIIDIR-IPN Sinaloa.

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