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RESEARCH NOTE

White Spot Syndrome Virus (WSSV) and Necrotizing Hepatopancreatitis (NHP) detection in wild shrimp of the San Andrés Lagoon, Mexico

Detección del Virus de la Mancha Blanca (WSSV) y Hepatopancreatitis necrotizante (NHP) en el camarón silvestre de la laguna San Andrés, México

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Abstract.- The presence of diseases caused by virus and bacteria pose a threat to the capture and commercialization of shrimp, and may cause significant economic damage. Nocturnal monthly sampling were conducted to detect the presence of IHHNV, WSSV, and NHP in San Andres Lagoon in Tamaulipas, Mexico, an important coastal ecosystem due to its shrimp fishery and the existence of shrimp farms in the area. Polymerase chain reaction (PCR) analysis in the shrimp tissue did not detect the presence of IHHNV, however, WSSV was detected, as well as NHP during July and August, when low salinities and high temperatures were recorded.

Key words: Shrimp, WSSV, NHP, IHHNV

INTRODUCTION

Shrimp fishery production ranks as the second place on production volume in Mexico, with the state of Tamaulipas as the first producer of wild shrimp in the Gulf of Mexico. Wild shrimp capture in this area (Tamaulipas and Veracruz coasts) consists mainly on the capture of *Farfantepenaeus aztecus*, with 95% of the total catch (INP 2006). Coastal lagoons in this area are rich in wild *F. aztecus* and *F. duorarum* populations; however, *Litopenaeus setiferus* can also be found in lagoons (Perez-Castaneda *et al*. 2010). These shrimp species show differential temporal abundance patterns in the lagoons (Wakida-Kusunoki *et al*. 2008).

Virus and bacteria are important pathogens that have been held responsible of disease in wild and cultured shrimp, especially when shrimp are under environmental stress. Pathogen prevalence in wild shrimp might represent a potential source of diseases for cultured organisms (Macías-Rodríguez *et al*. 2014), where prevalence and mortality can be very high due to the raised stress levels during culture, making disease outbreaks the main limiting factor for the development of the worldwide aquaculture industry (Dutta *et al*. 2015). Among the pathogens that have been identified as responsible of disease in shrimp aquaculture are the infectious hematopoietic necrosis virus (IHHNV), a widely distributed, highly prevalent and infective virus, considered one of the most serious problems affecting the shrimp farming industry (Vega-Heredia *et al*. 2012), and white spot syndrome virus (WSSV), deemed the most severe viral pathogen due to the high mortality it causes (Sanchez-Martinez *et al*. 2007). *Hepatobacterium penaei* is the causal agent of the necrotizing hepatopancreatitis (NHP) (Lightner *et al*. 2012), it is a small, Gram negative, intracellular, rickettsia-like bacteria, which was described for the first time in 1985 (Loy *et al*. 1996). This bacterium affects cultured shrimp in Latin America (Lightner & Redman 1994, Del Rio-Rodriguez *et al*. 2006) and Texas where it has caused losses of up to 90% of cultured shrimp (Lightner *et al*. 1992).

There are several coastal lagoons in Tamaulipas, of which the San Andres Lagoon forms a special ecosystem at the mouth of the Tigre River, an area with many *Litopenaeus vannamei* farms, an exotic species to the region. Transport of farmed shrimp to new culture areas may pose a risk to the local fisheries, since pathogens may spread to the wild through their byproducts or wastewater and shrimp escapes (del Rio-Rodriguez *et al*. 2006, Wakida-Kusunoki *et al*. 2011, Lightner *et al*. 2012). This makes the adequate diagnostics of disease in wild shrimp populations especially relevant, in order to implement strategies to avoid the introduction of the pathogens to the culture systems, as well as in the design of control methods. There are few studies on the viral and bacterial pathogens on wild shrimp populations in the Mexican gulf coast, therefore it is necessary to study their presence in wild shrimp, to help the local fishery; thus, the

purpose of this study was the screening of wild shrimp samples from the Tigre river - San Andres Lagoon area for IHHNV, WSSV and NHP using PCR.

MATERIALS AND METHODS

Shrimp were collected from San Andres Lagoon (22º19'49''-23º59'23''N; 97º45'40''-98º06'10''W) using a static artisanal fishing gear called 'charanga' from 4 sites in the lagoon. A charanga is a V-shaped trap (54 m mouth; 56 m long) made with stakes supporting a 3.81 cm mesh size net, with a rectangular enclosure at the tip end where shrimp are retained and harvested (DOF 1997)[1]. The sampling sites were based on the location of the charangas from local fishermen. Nocturnal samplings took place once a month during 12 months (January-December 2010). Twenty-four shrimp were randomly collected from each collecting site at each sampling time; 12 were used for PCR (preserved in EtOH 70%) and 12 for bacteriological studies (preserved on ice). Salinity and temperature were recorded for each site during sampling time using a multiparameter Mod.® (Yellow Spring Instruments, Ohio, USA).

For bacteriological studies, four-shrimp were pooled, macerated and seeded in TSA, MacConkey and TCBS culture media, and then incubated at 26 and 37ºC. Strain identification was performed using conventional biochemistry (Cowan & Steele 1993).

For PCR, four-shrimp were pooled: the hepatopancreas and pleopods from each shrimp in the pool were excised for DNA extraction, macerated using a surgical blade, and put in an Eppendorf tube. Viral and bacterial DNA was extracted using the Wizard SV Genomic DNA Purification System (Promega, USA). DNA amplification was performed using the following primers and thermal profiles. For the detection of WSSV, primers WS500F: CCTTCCATCTCCACCACACTT and WS500R: CTGTTCCTTGGCAGAGCATTC (Martorelli *et al*. 2010) were used. For IHHNV detection, primers 77012F: ATCGGTGCACTACTCGGA and 77353R: TCGTACT GGCTGTTCATC (OIE 2006) were used. For NHP, primers pf-1: ACGTTGGAGGTTCGTCCTTCA and pr-2: TCACCCCCTGCTTCTCATTGT (Loy *et al*. 1996) were used. The thermal profile used to detect WSSV consisted of an initial denaturation at 95ºC for 2 min, followed by 39 cycles of denaturation (95ºC, 30 sec), annealing (60ºC, 30 sec) and extension (72ºC, 1 min) with a final extension at 72ºC for 5 min. For the detection of IHHNV, an initial denaturation at 94ºC for 5 min, followed by 35 cycles of denaturation (95ºC, 30 sec), annealing (55ºC, 30 sec) and extension (72ºC, 1 min) with a final extension at 72ºC for 5 min was performed. Finally, to detect NHP an initial denaturation at 95ºC for 2 min, followed by 35 cycles of denaturation (95ºC, 20 sec), annealing (52ºC, 30 sec) and extension (72ºC, 30 sec) with a final extension at 72ºC for 4 min was done. All PCRs were performed in a total volume of 25 µl (12.5 µl of GoTaq 2x, 9.5 µl water, 1 µl of DNA, and 1 µl of each primer (50 pmol). Three microliters of the amplified product were analyzed by a 1% agarose gel electrophoresis (100V per 90-120 min), and visualized using a DIGI DOC-IT System.

Statistical analyses to determine the dependence of the virus and bacteria upon salinity levels and temperature were performed by using contingency tables χ^2, using the software Statistica v.6 (StatSoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

The highest salinity levels were recorded in April (33.08), while the lowest salinity level was recorded in July (3.3). The highest water temperature was recorded in August (33.9ºC) and lowest water temperature in December (19.3ºC). The shrimp species found were identified as *L. setiferus* and *F. aztecus*. In previous studies in other coastal lagoons in Tamaulipas (Aguirre-Guzmán *et al*. 2010), the presence of at least 3 shrimp species has been reported: *F. aztecus*, *F. duorarum*, and *L. setiferus*. In this study, the dominant shrimp species was *L. setiferus* (320 organisms), followed by *F. aztecus* (24 organisms). No *F. duorarum* specimens were recorded.

The main bacteria genera identified by bacteriology corresponded to *Aeromonas* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Bacillus* sp., *Vibrio* sp., and *Micrococcus* sp. The bacteria present in wild shrimp with the highest frequency were *A. hydrophila*, *Bacillus* sp. and *Vibrio* sp., which are considered part of the normal bacterial flora of the shrimp. These bacteria are frequently reported from shrimp, and their presence is probably due to contamination in the lagoon.

Using PCR, WSSV positive wild shrimp were detected in the organisms sampled in November. All other shrimp samples, at all other sampling times were negative for this virus. The results also show that wild shrimp were negative for IHHNV at all sampling times at San Andres Lagoon, whereas NHP was detected during July and August, with negative samples at all other sampling times. There are few studies in the Gulf of Mexico related to the presence of bacteria and virus on wild shrimp,

[1]Diario Oficial de la Federación (DOF). 1997. AVISO por el que se da a conocer la autorización para utilizar charangas como equipos de pesca para la captura de camarón en los sistemas lagunarios estuarinos de Tamaulipas y del norte de Veracruz. <http://dof.gob.mx/nota_detalle.php?codigo=4901449&fecha=21/11/1997>

however the presence of NHP in wild shrimp has been previously confirmed (Aguirre-Guzmán *et al*. 2010). To our knowledge, this is the second record of NHP on wild shrimp in Tamaulipas coastal lagoons.

Regarding IHHNV, these results agree with other studies, which have not detected IHHNV in wild shrimp populations in other coastal systems in the Gulf of Mexico, such as Laguna Madre in Tamaulipas (Aguirre-Guzmán *et al.* 2010), the Jamapa river in Veracruz (Domínguez-Machín *et al.* 2011), and the Laguna de Términos in Campeche (del Río-Rodríguez *et al.* 2013). There is one report of the detection of IHHNV in wild *L. setiferus* and *F. aztecus* from an estuary south of the Laguna Madre lagoon in Tamaulipas (Guzman-Saenz *et al.* 2009). The non-detection of IHHNV-positive shrimp may suggest that either the wild shrimp populations from the San Andrés Lagoon are still free of this virus or its prevalence is very low. A study on *L. vannamei* farmed near the San Andrés Lagoon reported the absence of IHHNV in the farmed shrimp (Gutiérrez-Salazar *et al*. 2011); conversely, a more recent study by López-Téllez *et al*. (2015) stated the presence of IHHNV in farmed *L. vannamei*, which originated from a single shrimp hatchery, in several shrimp farms near the San Andrés Lagoon; however, this study did not include wild shrimp species in the nearby lagoon. These differences with our results may be explained by the fact that in our study, we only included native wild shrimp (*L. setiferus* and *F. aztecus*), whereas López-Téllez *et al.* (2015) focused exclusively on cultured *L. vannamei* (an exotic species from the Pacific), coming from a single shrimp hatchery. According to these authors, a potential source of IHHNV in shrimp farms in Tamaulipas could be the introduction of *L. vannamei* postlarvae from the Pacific coast, where the IHHNV was initially introduced from the Philippines in the 1980s (Lightner 2011). Even though we did not find the presence of IHHNV in wild shrimp during our study, the infection with this virus in native wild shrimp populations of the Gulf of Mexico via the introduction of *L. vannamei* postlarvae from the Pacific is a latent possibility.

Statistical analysis by contingency tables determined that the presence of WSSV in the shrimp by PCR is dependent ($P < 0.001$) on the salinity levels and temperature (Tables 1 and 2), *i.e*., there is direct influence of both salinity and temperature in the presence of this virus. In fact, positives for this virus were obtained at salinities of 25 and temperatures of 26ºC. Similarly, WSSV outbreaks in the cultured shrimp *Penaeus monodon* occurred when water temperature ranged from 26 to 34°C (Tendencia & Verreth 2011). With respect to NHP the results show that the presence of NHP was independent ($P= 0.28$) of salinity levels and temperature, and therefore these variables do not influence the presence of NHP (Tables 3 and 4).

Table 1. WSSV positive and negative samples according to different salinity levels. The result of the independence test was $\chi^2= 15.13$ ($P< 0.001$) / Muestras positivas y negativas a WSSV de acuerdo a los diferentes niveles de salinidad. El resultado de la prueba de independencia fue $\chi^2= 15,13$ ($P< 0,001$)

Salinity	Low 10	Medium 25	High 31	Total
Positive (+)	0	6	0	6
Negative (-)	36	18	20	74
Totals	36	24	20	80

Table 2. Relation of positive and negative samples to WSSV according to different temperature levels. The result of the independence test was $\chi^2= 15.13$ ($P< 0.001$) / Relación de las muestras positivas y negativas para WSSV de acuerdo a los diferentes niveles de temperatura. El resultado de la prueba de independencia fue $\chi^2= 15,13$ ($P< 0,001$)

Temperature	Low 20ºC	Medium 26ºC	High 31ºC	Total
Positive (+)	0	6	0	6
Negative (-)	20	18	36	74
Totals	20	24	36	80

Table 3. NHP positive and negative samples according to different salinity levels. The result of the independence test was $\chi^2= 2.50$ ($P= 0.28$) / Muestras positivas y negativas a NHP de acuerdo a los diferentes niveles de salinidad. El resultado de la prueba de independencia fue $\chi^2= 2,50$ ($P= 0,28$)

Salinity	Low 10	Medium 25	High 31	Total
Positive (+)	2	0	0	2
Negative (-)	34	24	20	78
Totals	36	24	20	80

Table 4. NHP positive and negative samples according to different temperature levels. The result of the independence test was χ^2= 2.50 (*P*= 0.28) / Muestras positivas y negativas a NHP de acuerdo a los diferentes niveles de temperatura. El resultado de la prueba de independencia fue χ^2= 2,50 (*P*= 0,28)

Temperature	Low 20ºC	Medium 26ºC	High 31ºC	Total
Positive (+)	0	0	2	2
Negative (-)	20	24	34	78
Totals	20	24	36	80

High market demand results in shrimp farms being located in the vicinity of coastal lagoons, where wild shrimp are found. Both shrimp groups (cultured and wild) are susceptible of infectious disease and water may act as a vehicle for the distribution of pathogens. Salinity and temperature fluctuations may induce environmental stress in shrimp making them more susceptible to disease. The temperature recorded in the study area showed monthly increments according to the season in all 4 sampling sites with December and January registering the coldest temperatures, and the highest temperatures from May to August. Salinity levels decreased during July, August and September, because this is a rainy season in the study area and there is more freshwater arriving in the lagoon.

The presence of WSSV and NHP in wild shrimp from San Andrés Lagoon signals the risk of the proximity of wild shrimp populations and shrimp farms, which are adjacent in the Gulf of Mexico coast, especially since most of the shrimp farms in Tamaulipas are located in this area.

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