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Epidemiological status of Taura syndrome and Infectious myonecrosis viruses in *Penaeus vannamei* reared in Pernambuco (Brazil)

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Abstract

Several viral diseases of cultured *Penaeus vannamei*, threaten the sustainability of shrimp farming industries worldwide. Taura syndrome virus (TSV) is considered one of the most harmful pathogens in the Americas, with cumulative mortalities reaching 40 to 95%. Another virus, named Infectious myonecrosis virus (IMNV), was first identified in *P. vannamei* reared in Northern Brazil in 2004. In this study, we investigated the epidemiological status of TSV and IMNV in cultured shrimp by reverse transcriptase-polymerase chain reaction (RT-PCR) in Pernambuco state. Eleven commercial farms were randomly sampled between August and December of 2004, out of 14 existing farms in the state. Within each farm, ponds were randomly sampled and 10 shrimps were tested within each of them. A total of 505 individuals samples were RT-PCR tested. None was positive for TSV, suggesting that the virus was not present in the study population, which is consistent with the absence of clinical reports. Nine out of 11 farms were positive for IMNV, which should be considered alarming and strengthens the need for establishing monitoring programs at the national level. These results provide new insights into the epidemiological status of both diseases and may contribute to the development of a national biosecurity plan.

Keywords: *Penaeus vannamei*; TSV; IMNV; RT-PCR; Epidemiological status

1. Introduction

Shrimp production has grown significantly over the past 20 years, comprising 20.1% of total global aquaculture by value in 2002. In 1989, the first major crash in the production of farm-raised shrimp occurred in Asia. Further crashes in production have subsequently characterized the world's shrimp farming industry, largely due to viral diseases (FAO, 2004).

For any aquatic species, the transition from life in a wild environment to controlled monoculture systems can be accompanied by several changes, such as increased population densities, frequent degradation of the culture system's environment, the mixing of populations of different origin, etc. These changes increase the probability of outbreaks of serious diseases.

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Several viral diseases have occurred in the Americas, threatening the sustainability of the shrimp farming industry. Among the most important diseases are those caused by Taura syndrome virus (TSV), White spot syndrome virus (WSSV) and Infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Lightner and Redman, 1998; Lightner, 1996; GAA, 1999a,b).

Taura syndrome emerged as a new disease in 1992 on shrimp farms near the Taura River in the Gulf of Guayaquil, Ecuador (Jimenez, 1992) and spread rapidly in Latin America with cumulative mortalities of 60 to>90% (Lightner, 1999). TSV became one of the most pressing disease issues in shrimp culture, with recent outbreaks occurring in 2005 in Venezuela (OIE, 2005). Lightner (2003) estimates that TSV has led to a direct economic loss to shrimp farm production in the Americas of US$ 1 to 1.3 billion.

Another virus, named Infectious myonecrosis virus (IMNV) was first identified in *Penaeus vannamei* reared in Northern Brazil in 2004 (Lightner and Pantoja, 2004). The first documented occurrence of Infectious myonecrosis disease (IMN), caused by IMNV, was in a shrimp farm in the state of Piauí (Northeast, Brazil) in 2002, however during the rainy season in the months of January through March of the following year, the disease spread to other adjoining states. In 2004, most of the shrimp farms of the northeast states were infected by IMNV, including the state of Pernambuco. Mortality rates ranged from 35 to 55% in 12 g shrimp and the economic loss was estimated to be US$20 million in 2003 (Nunes et al., 2004).

Disease control has become a priority in responsible shrimp farming. According to Bachère (2000), the durability of the shrimp production industry is dependent on the equilibrium between environmental quality, prevention of diseases by early diagnostic as well as epidemiological surveys of the pathogens, and assessment of shrimp health status.

For these reasons, this study was conducted to gain insights into the epidemiological status of two shrimp viruses, TSV and IMNV, in farms of Pernambuco, by reverse transcriptase-polymerase chain reaction (RT-PCR) based diagnosis. The results presented here are extremely relevant and can contribute to the development of a national biosecurity plan.

2. Material and methods

2.1. Sampling methods

Sampling was carried out from August to December 2004 in 6 different counties, in the southern and northern zones of Pernambuco state (Northeast, Brazil), comprising 11 farms out of a total of 14 existing shrimp farms, according to FAEPE (Federação da Agricultura do Estado de Pernambuco). The objective was to conduct a census of farms, in order to establish the epidemiological status and provide a baseline assessment for future planning. Unfortunately, three farms could not be visited out due to logistical problems. Within each farm, a two-stage random sample was carried out with a view to classifying the farm status (infected/non infected). The first stage was a random sample of ponds and the second stage was the testing of a predetermined number of individual shrimp samples per pond. For each pond a cast net was thrown three times in different points, then shrimp were pooled together in a bucket and 10 individuals were randomly harvested from it. The number of individual shrimps tested per pond (10) was calculated according to the following formula (Cannon, 2001):

$$n \cong \frac{\left\lfloor \left(1-(1-\alpha)^{1/D}\right)(N-1/2(\mathrm{Se}D-1)) \right\rfloor}{\mathrm{Se}}$$

where:

n	sample size
α	confidence level = 0.95
N	number of individuals at risk = 100,000 or infinite population
D	minimum number of infected individuals or threshold prevalence = 30% of at-risk population
Se	sensitivity test = 0.9

In two farms, 15 samples were taken, thus raising the confidence level, for the same threshold prevalence. The latter parameter, fixed at 30%, is based on the assumption that, for both diseases, there are no specific monitoring and control programs and, therefore, an infected pond would have a large proportion of infected shrimps, as these are fast moving diseases. The test sensitivity was fixed at 90% in order to avoid the assumption of perfect test performance.

The same formula was used to calculate the number of ponds to be sampled per farm. In this case, the threshold prevalence was set at 50%, as both infections are likely to affect a high number of ponds, should the farm be infected. The sensitivity was 95%, which was the confidence level used for the shrimp sample. It should be noted that in some farms the number of sampled ponds was well above the minimum number necessary for achieving the predetermined confidence level. Overall, a total of 48 ponds and 505 shrimps were sampled, as shown in Table 1.