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FIRST REPORT OF *White spot syndrome virus* IN FARMED AND WILD PENAEID SHRIMP FROM LAGOA DOS PATOS ESTUARY, SOUTHERN BRAZIL

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ABSTRACT

In this study, we detected *White spot syndrome virus* (WSSV) in wild *Farfantepenaeus paulensis* collected in the Lagoa dos Patos estuary and cultivated *Litopenaeus vannamei*. This is the first report of WSSV in *F. paulensis* from Lagoa dos Patos and farmed *L. vannamei* shrimps in Rio Grande do Sul.

Key words: WSSV, viral diseases, *Farfantepenaeus paulensis*, *Litopenaeus vannamei*, epizootiology.

White spot syndrome virus (WSSV) is a double stranded DNA virus, which belongs to the family *Nimaviridae*, genus *Whispovirus* (17). WSSV has become the most threatening infectious agent in shrimp aquaculture (7). Estimated world economic losses associated to WSSV approach one billion US dollar per year since its first report in 1992 (8). The disease gross signs include decrease feed consumption, lethargy, white inclusions in the cuticle and reddish to pink discoloration (6).

Epidemiological surveys have evidenced the widespread distribution of WSSV in wild shrimp around the world (2, 3, 15, 18, 21, 22). In Brazil, the first notification of WSSV in shrimp farms occurred in 2005, in the states of Santa Catarina (South) and Ceará (Northeast). Cavalli *et al*. (2) also found WSSV infected shrimp of different species in offshore waters of Santa Catarina coast, indicating that the virus is wide spread in the Southern Atlantic Ocean.

The continental shelf of the extreme Southern Brazil is one of the most productive fishing areas in the country (19). Great part of the commercial seafood is harvested from the Lagoa dos Patos estuary located near Rio Grande city, in Rio Grande do Sul state. The pink shrimp *Farfantepenaeus paulensis* is the main fishing product caught from the Lagoa dos Patos (5). In Rio Grande do Sul, the catches of pink shrimp increased from 2,274 tones in 2006 to 3,866 tones in 2007 (11), being an important business to the fishermen community.

Besides, there is a small number of *Litopenaeus vannamei* farms in operation. These have played an increasingly prominent role on the regions´s socioeconomic status. As WSSV causes increase mortality in population, the infection of wild and raised shrimp could represent a heavy burden on fishing and aquaculture activities in this area.

No searches have been carried out so far for WSSV in wild or captive shrimp in the state of Rio Grande do Sul. Here we present a preliminary report on the occurrence of WSSV in wild shrimp in the Lagoa dos Patos estuary, as well as in some shrimp farms in the region. Thus, specimens of *Farfantepenaeus paulensis* (n=450) from the Lagoa dos Patos estuary were randomly collected in three different sites (Fig. 1,

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Table 1), as well as 441 *Litopenaeus vannamei* specimens from five local shrimp farms (Fig. 1, Table 1). The samples were collected between January and March 2008. All specimens were transported to the laboratory preserved in 95 % ethyl alcohol. DNA extractions were performed with DNAzol (Invitrogen), according to the manufacturer's instructions. Diagnostic by PCR was carried out by nested-PCR as described by Lo *et al*. (14). The PCR products were resolved in 1 % agarose gel, stained with ethidium bromide and viewed on a UV transilluminator. All positive cases were repeated at least twice. Partial WSSV DNA of PCR positive samples were sequenced using the automatic sequencer MegaBACE 500 (GE Healthcare Life Sciences, Brazil). The sequences were compared with other WSSV sequences of equivalent genomic regions available at GenBank with the BLASTn on-line tool (1).

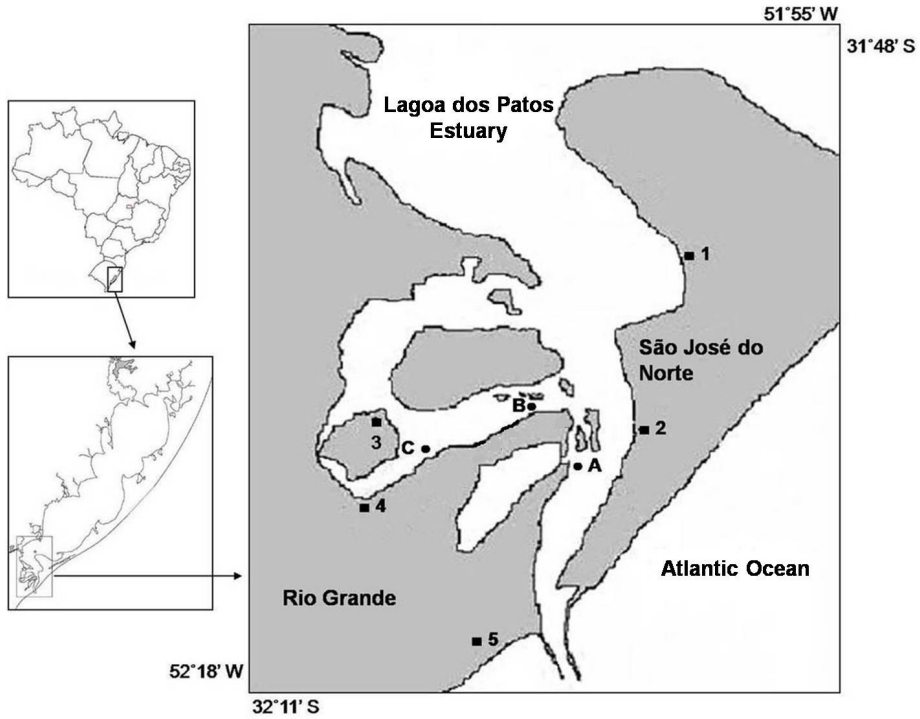

Figure 1. Lagoa dos Patos estuary, indicate the samples colection in environmental and shrimp ponds. The letters (●A,B,C) presents samples sites where wild *Farfantepenaeus paulensis* were collected from estuary. The numbers (■1-5) shown the localization of shrimp farms where *Litopenaeus vannamei* were sampled.

Table 1. Number of shrimps collected in *Lagoa dos Patos* estuary (estuary A, B, C) and shrimp farms (1-5) and positive cases of WSSV by nested-PCR.

Sampling Site	Collected shrimps	Infected shrimps	Species
Farm 1	65	60	*L. vannamei*
Farm 2	32	28	*L. vannamei*
Farm 3	74	15	*L. vannamei*
Farm 4	160	70	*L. vannamei*
Farm 5	114	113	*L. vannamei*
Estuary A	150	35	*F. paulensis*
Estuary B	150	10	*F. paulensis*
Estuary C	150	0	*F. paulensis*

For histopathological analysis, the shrimps were transferred to Bouin's solution for 24 hours. Samples were processed for standard histological methods (20), stained with hematoxylin, eosine and Periodic Acid Schiff's (PAS).

Based on PCR analysis, it was detected the presence of WSSV in wild animals of the Lagoa dos Patos estuary, in two sites (A and B, Fig. 1). Among the 150 *Farfantepenaeus paulensis* collected in each locality, were detected 35 WSSV infected shrimps at site A and 10 infected shrimps at site B (Table 1). The infected *Litopenaeus vannamei* was detected in all five shrimp farms (Table 1). This positive results were confirmed by sequenced genome fragments and showed high nucleotide similarity with WSSV sequences available at GenBank (*E value* = 0). Also, histopathological lesions revealed typical basophilic inclusions in tissues of *F. paulensis* from estuary.

This is the first report on the occurrence of WSSV in *F. paulensis* from the Lagoa dos Patos and *L. vannamei* from shrimp aquaculture in the state of Rio Grande do Sul, Brazil. The stocks of shrimps from lagoons in Rio Grande do Sul are originated from Atlantic Ocean waters, where infected wild shrimps were reported (2). Hence, the most likely source of the virus would clearly be infected wild stocks from Atlantic waters. WSSV spreads rapidly along the Atlantic Ocean carried in infected animals by natural currents. This is shown by WSSV presence in Northern Atlantic Ocean, USA (3), Southern Atlantic Ocean, Brazil (2) and the most Austral occurrence in Bahia Blanca Estuary, Argentina (16).

However, in agreement with Vaseeharan *et al*. (22), the number of infected shrimps was higher in wild animals collected next areas used for shrimp production. Water sources from aquaculture could contribute to the spread the pathogen. As well as the exposure wild penaeid shrimp to the virus depends on the patterns of viral entry into marine systems, the movements patterns of the shrimp migration (12) and also on the water currents.

Besides, the impact of WSSV infection on wild stocks seems to be little significance on the shrimp population dynamics. Even though WSSV infected wild shrimp, the statistical fishery data of pink shrimp ranged from 1,296 tones in 2004 to 2,274 tones in 2006 (9, 10), showing no significant effect on the shrimp population. Statistical fishery data suggests that a little variation in fish catches is more related to environmental factors (4, 5) than viral disease episodes. Nevertheless, the evidence of virus impact on population requires data before, during and post epizootic event (13). In this way, is necessary an epidemiological research to evaluate the possible impact of white spot syndrome in shrimp population.

This work presents the first report of WSSV in wild shrimp in Rio Grande do Sul (Southern Brazil), increasing the occurrence of this virus in South America, which is an important data related to the virus spread and biogeography. It should be noted that this is important information for future studies of viral global mapping and distribution, especially with regard to the natural environment.

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