Taura syndrome in México: follow-up study in shrimp farms of Sinaloa

Martha Zarain-Herzberg a,*, Felipe Ascencio-Valle b

a Centro de Ciencias de Sinaloa, Culiacán, Sinaloa 012121, Mexico
b Centro de Investigación Biológica del Noroeste, La Paz, Baja California 021121, Mexico

Received 8 March 2000; received in revised form 30 June 2000; accepted 3 July 2000

Abstract

The Taura syndrome (TS) is one of the viral shrimp diseases that has most affected cultivation of the shrimp Litopenaeus vannamei in America. We analyzed the presence of TS, from its first detection in May 1995 in 75% of the shrimp farms of the Guasave district in Sinaloa State, México, and its progressive spreading from the north toward the central and southern zones of the state, to the districts of Navolato and Elota. The main histopathological findings in shrimp tissues analyzed were necrotic areas in the cuticular epithelium, near the sites where there is melanization, and in various parts of the surface of the body, appendages, gills, hindgut, esophagus, and stomach. As revealed with hematoxylin and eosin–phloxine staining, the cuticular lesions were generally spherical and with cytoplasmic inclusion bodies. Some of the affected cellular nuclei of the tissues were pycnotic or karyorrhectic, giving a “buckshot” appearance to the lesions. During 1996, shrimp samples were collected from 33 shrimp farms in different areas of Sinaloa. The histopathological analysis revealed a 92% prevalence of TS in the central zone, 78% TS in the southern zone, and 73% TS in the northern zone. In 1997, histopathological analysis of shrimp samples collected in the same shrimp farms revealed a decrease in TS. In 1998, a significant reduction in the prevalence of TS was observed, with only 30% incidence of TS in the farms sampled. We concluded that the epidemic of TS in shrimp farms of Sinaloa reached a peak in 1996, followed by a steady decline that closely paralleled the switch by the industry from culturing TS virus TSV-susceptible L. vannamei to TSV-resistant L. stylirostris. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Penaeid shrimp; Litopenaeus vannamei; Taura syndrome virus (TSV); Viral disease
1. Introduction

Aquaculture is an economically important activity in the state of Sinaloa, México. The Pacific Ocean coast of Sinaloa has 16 important lagoon systems. Adjacent to the coastal lagoons are natural lands of about 250,000 ha suitable for aquacultural development. At present, there are about 220 shrimp farms in Sinaloa with an estimated production of 13,000 tons/year (Rosenberry, 1998), representing 76% of the national shrimp production.

The Taura syndrome (TS) is one of the most economically significant diseases that affects the cultivation of the shrimp species *Litopenaeus vannamei* in America. TS has traveled progressively from farms of the Gulf of Guayaquil, Ecuador to farms of Peru, Colombia, Honduras, United States, and México (Wigglesworth, 1994; Lightner, 1996a; Zarain, unpublished data). The penaeid shrimp species that is most affected by this disease is *L. vannamei*, with a cumulative mortality of 50–90% in aquaculture or in the wild (Brock et al., 1995; Overstreet et al., 1997).

TS is a disease of the nursery phase of the shrimp, affecting mainly small juveniles with body weight between 0.05 and 5 g (Lightner and Redman, 1994; Brock et al., 1995).

In this work, TS was diagnosed in shrimp farms by routine histopathological examination and hybridization in situ. The histopathological alterations in TS-positive shrimp include multifocal necrotic areas in the cuticular and subcuticular epithelium, characterized by the presence of basophilic and eosinophilic inclusion bodies, imparting a “buckshot” appearance to affected tissues. TS can be distinguished by three overlapping clinical phases that are histologically different (Lightner et al., 1995; Lightner, 1996a; Hasson et al., 1999): (a) a peracute–acute phase, characterized by moribund shrimp that show a general pale-reddish coloration caused by the expansion of red chromatophores. In this phase, the shrimp commonly die during the molting process; (b) the shrimp that survive enter a transition phase (or a recovery phase) that may evolve to a chronic phase, which is distinguished histologically by multifocal-melanized lesions in the cuticular epithelium, and (c) a chronic phase where the shrimp resume apparent normal morphology and development patterns.

Because the economic loses that TS represents in the shrimp farms of Sinaloa, we established a TS-monitoring program in the shrimp farms throughout Sinaloa State from 1995 to 1998 to follow the progress of the TS epizootic.

2. Materials and methods

2.1. Areas studied

The State of Sinaloa is in north western Mexico with coordinates lat 27°29' N and long 105°23' E to 109°27' W.

The northern study zone included the coastal districts of Ahone and Guasave. The central zone included the districts of Angostura, Navolato, and Culiacán. The southern zone included the districts of Elota, San Ignacio, Mazatlán, Rosario, and Escuinapa (Fig. 1).
2.2. Sample collection

Shrimp production in Sinaloa is done in two cycles per year, the first from March to June (the spring–fall cycle), and the second July to December (the autumn–winter cycle). Shrimp samples were collected during each of the two cycles in 1995, 1996, 1997 and 1998. Initially, to determine the exact location of TS in shrimp farms of the state of Sinaloa, we sampled 14 shrimp farms in the northern, central, and southern zones of the state from March to April of 1995. The second sampling in 1995 was done in May and included 15 shrimp farms belonging to the indicated zones (Table 1). In 1995 and 1996, the presence of the disease was determined using staining with hematoxylin and eosin–phloxine (H&E) as the main criteria for diagnosis. The shrimp examined in 1997 and 1998 were analyzed by histology (H&E) as well as by the use of a commercial TS virus (TSV) genomic probe analysis kit, confirming the histological results (not shown).

_L. vannamei_ showing characteristic signs for TS (Lightner et al., 1995) were collected from farms and transported live in plastic containers to arrive at the laboratory, within 8 h of collection.
2.3. Histopathological analysis

When the shrimp samples reached the laboratory, the shrimp were preserved in Davidson’s fixative solution and were transferred to 50% ethanol after 48 hours (Bell and Lightner, 1988, Hasson et al., 1997). For histological analysis, the conventional staining technique with hematoxylin and eosin–phloxine (H&E) was used (Bell and Lightner, 1988).

2.4. In situ hybridization

Shrimp samples preserved in R-F Davidson’s fixative solution for 48 hours were transferred into 50% ethanol (Bell and Lightner, 1988; Hasson et al., 1997). For hybridization in situ, the TS Shrimp probe cDNA corresponding to the sequence located at positions 5’-345 to 3’-657 of the TSV (Mari et al., 1998) was used following the methodology recommended by the manufacturer (DiagXotics, Wilton, CT, USA).

3. Results

The typical histological signs found in the shrimp analyzed that had TS were necrotic areas with nuclear pycnosis and karyorrhexis of the cuticular epithelium and the presence of cytoplasmic inclusion bodies producing the distinctive “peppered” or “buckshot” appearance in various parts of the surface of the body, appendages, gills, hindgut, esophagus and stomach. Diseased shrimp were lethargic, and there was an expansion of red chromatophores over the entire body, especially in the antennae, pleopods, periopods, and uropods, and the soft cuticle. The affected shrimp had melanized cuticular lesions (Hasson et al., 1995; Hasson et al., 1999; Lightner et al., 1995).

In February 1995, the Mexican Government reported the presence of TS in wild shrimp captured at the border of Mexico with Guatemala, but without specifying the
exact locations. There was no evidence of the TS disease using the standard histopathological H&E technique in samples collected March to April 1995 (data not shown).

In the sampling in May, 75% of shrimp from the northern zone (a body weight range of 0.3–12.3 g) showed histological signs of the disease. In the sampling in June, TS lesions were detected in 100% of the shrimp farms examined in the central zone, in shrimp specimens with a weight range similar to the shrimp infected in May. In October, all of the shrimp farms examined in the southern zone also showed the presence of TSV (Table 2). In all the farms studied in May, June, and October, the only species cultivated was *L. vannamei*.

In 1996, the prevalence of TS was greatest in the central zone of the state (92%), followed by the southern zone (78%), and the northern zone (73%) (Table 3). The shrimp species cultivated in the farms studied was *L. vannamei*, both of hatchery and wild origin. The prevalence of TS in 1997 and 1998 was lower in the northern and southern zones, compared to that found in 1996.

The shrimp collected at the farms for histological and TSV genomic probe were found in various developmental stages, as reflected by the wide range of body weight of shrimp infected with the TSV.

In 1998, the prevalence of TS was also lower for the central zone, compared to 1996 and 1997, with the mixed origin of *L. vannamei* species similar to 1997. *Litopenaeus stylirostris* is the most commonly farmed species (Table 3).

In Fig. 3, the decrease in the relative percentage of cultivation of *L. vannamei* from 100% in 1995, to 30% in 1998 is shown. However, we did not detect a clear relation of the presence of TS with the origin of the seed.

### Table 2
Prevalence of TS in shrimp farms, in the State of Sinaloa by zone, from May to October 1995

<table>
<thead>
<tr>
<th>Zone</th>
<th>Prevalence percent</th>
<th>Month†</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Guasave</td>
<td>(6/8) 75</td>
<td>May</td>
</tr>
<tr>
<td>Center Navolato</td>
<td>(4/4) 100</td>
<td>June</td>
</tr>
<tr>
<td>South Elota</td>
<td>(3/3) 100</td>
<td>October</td>
</tr>
</tbody>
</table>

†Month in which TSV was found for the first time in the corresponding district.

### Table 3
Prevalence of the TS in shrimp farms, in the State of Sinaloa, sampling from 1996 to 1998 by zone

<table>
<thead>
<tr>
<th>Year</th>
<th>Zone</th>
<th>Prevalence percent</th>
<th>Origin of <em>L. vannamei</em> percent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cultivated</td>
<td>Wild</td>
</tr>
<tr>
<td>1996</td>
<td>North</td>
<td>(8/11) 73</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Center</td>
<td>(12/13) 92</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>(7/9) 78</td>
<td>83</td>
</tr>
<tr>
<td>1997</td>
<td>North</td>
<td>(3/9) 34</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Center</td>
<td>(7/9) 78</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td>South</td>
<td>(1/5) 20</td>
<td>40</td>
</tr>
<tr>
<td>1998</td>
<td>Center</td>
<td>(8/23) 34</td>
<td>30</td>
</tr>
</tbody>
</table>
4. Discussion and conclusions

There were six viral diseases that had been recognized before to 1992 (Lightner and Redman, 1992). Now, more than 20 viruses that affect penaeid shrimp are known (Lightner, 1996a). The viruses that have most affected shrimp farms in America are Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) and TSV. Recently, the viruses White Spot Syndrome Virus (WSSV) and Yellow Head Virus (YHV) have been found in the western hemisphere (Nunan et al., 1998). WSSV has caused severe effects on shrimp production of several countries in Central America (Alday, 1999).

In February 1995, the Mexican Fisheries Ministry (SEMARNAP) reported the presence of TSV in wild-type shrimp captured on the border of Mexico with Guatemala, although the exact location of the disease episode was not determined. The goal of our work was to establish if TS was also affecting the shrimp farms of Sinaloa. From May to October of 1995, we screened for the presence of the TS, in the district of Guasave in Sinaloa, and found that the TS disease was spreading from the north towards the southern regions of the state, to the districts of Navolato and Elota (Table 2 and Fig. 1). The rapid spread of TS in Sinaloa may have been caused by factors, such as (1) movement of TSV-infected postlarvae (PL) and brookstocks in Sinaloa. This also happened in Taiwan (Chien et al. 1999), (2) the contribution of other transmission vectors as sea gulls or aquatic insects (Lightner et al., 1995), (3) other dissemination factors of the spread of the TS could have been oceanic currents, this being less probable because under normal climatic conditions the circulation of the main oceanic currents of the North Pacific Ocean and the California Gulf flow westward (Bowden, 1965). In 1996, we detected, with high incidence, the presence of TS in shrimp farms sampled in the northern, central, and southern zones of Sinaloa state. Fortunately, there was a major reduction in the number of infected farms in 1997 and 1998 in the three zones. Nevertheless, the central zone of Sinaloa still continues to have infected shrimp farms and follow-up investigations are in progress. (Fig. 2).

As a result of TS, the aquaculture shrimp production of Sinaloa State decreased in 1996, according to official production reports (SEMARNAP, 1996), compared to 1995. Production increased almost 45% in 1995, even though in that year the presence of TS was detected. In 1996, the shrimp production decreased 37%, though in other countries the production had been reduced more severely (Lightner, 1996b). In 1997, the production recovered to that of 1995. In 1998, the production reached 13,500 tons, an increase of 50% from 1997.

We concluded the TS epidemic in shrimp farms of Sinaloa state reached a peak in 1996 and there has been a steady reduction in the number of shrimp farms infected, with a stabilization in the production of the shrimp farms of Sinaloa by 1998. This stabilization was possibly because of the change in species cultivated (Fig. 3) from *L. vannamei* to *L. stylirostris*.

The decrease of TSV from 1995 to 1998 with respect to the decrease of the percentage of *L. vannamei* cultivated in these years has a correlation of $r = 0.98$ (Figs. 2 and 3), indicating these two variables are closely related. The data in Fig. 3 show the percentage of *L. vannamei* cultured in Sinaloa decreased from 100% in 1995 to $\sim 30\%$ by 1998. This significant reduction in TSV-susceptible hosts must have been a major
factor in the decrease in prevalence that it nearly parallels (Fig. 2). Experimentally infected *L. stylirostris* PL and juveniles with TSV are resistant to disease (Brock et al., 1995; Lightner, 1996a; Overstreet et al., 1997). Perhaps, as a consequence of reducing
the number of susceptible hosts in Sinaloa in 1996–1998 (i.e. *L. vannamei*), the prevalence of TS also decreased. This measure may have helped to reduce the number of TSV epizootics in Sinaloa and possibly its eventual eradication.

Probably, taking together, these results also indicate a difference in susceptibility of this shrimp variety to the TSV (Overstreet et al., 1997), when the species were cultivated in the particular environmental conditions (temperature, salinity) found in the shrimp farms of Sinaloa.

We also conclude, that the tolerance of *L. vannamei* to the TSV did not increase with the size of the shrimp, as previously reported by Lotz (1997), because the body weight of infected shrimp in Sinaloa showed a very wide range, from 0.063 to 15.0 g.

Acknowledgements

Funding for this research was provided by Secretaría del Medio Ambiente Recursos Naturales y Pesca (SEMARNAP). Dirección General de Acuacultura (DGA) of the Mexican Federal Government.

We would like to thank M.S. Jorge Gastélum-Escalante and Dr. Angel Zarain-Herzberg for the critical reading of this manuscript. We also thank Jorge Salcido, Silvia López, Rosa Raygoza, Celina Villegas, Vanía Fueyo, and Jorge Navarro for their expert technical assistance.

Special thanks to Dr. Donald Lightner for valuable commentaries and critical reading. Thanks to Dr. Ellis Glazier for editing the English-language text.

References


