

NOTE

# Hyperthermia does not protect Kona stock *Penaeus vannamei* against infection by a Taura syndrome virus isolate from Belize

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**ABSTRACT:** This study evaluated the susceptibility of *Penaeus vannamei*, Kona stock-line, to infection by an isolate of Taura syndrome virus from Belize (TSV-BZ) under hyperthermic conditions (32°C). Shrimp exposed to the reference Hawaii-94 isolate of TSV (TSV-HI) showed resistance to infection at 32°C as demonstrated by the absence of mortality, histopathological lesions and decreased viral load by qPCR. However, at 32°C, shrimp were fully susceptible to the disease caused by TSV-BZ, exhibiting high mortality, severe histopathological lesions and increased viral load. This susceptibility of shrimp to TSV-BZ infection under hyperthermic conditions was independent of the route of infection (injection vs. *per os*) and the salinity of the water (11 vs. 28). TSV-BZ might be a temperature-permissible mutant of TSV.

**KEY WORDS:** Belize variant · Taura syndrome virus · Immune response · Hyperthermia · *Penaeus vannamei* · Shrimp

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## INTRODUCTION

Taura syndrome virus (TSV) is a shrimp virus and a member of the *Discistoviridae* family (Hasson et al. 1995, Mayo & Ball 2006). Many isolates of the virus have been characterized, notably the Hawaii-94 reference strain (TSV-HI) and more recently the 2002 isolate from Belize (TSV-BZ) (Erickson et al. 2005).

Montgomery-Brock & Shimojo (2003) showed that hyperthermic water temperature (32°C) was associated with higher survival of shrimp exposed *per os* to TSV-HI. In the present study, we report the results of experiments that showed Kona stock *P. vannamei* exposed to TSV-BZ presented unexpectedly high mortalities under hyperthermic conditions.

## MATERIALS AND METHODS

**Shrimp stock.** The Oceanic Institute in Hawaii provided specific pathogen free (SPF) *Penaeus vannamei*, Kona stock-line. Shrimp weighed between 1 and 4 g.

**Bioassay.** For all bioassays, 90 l aquaria fitted with a biological filter were used as described in White et al. (2002). Artificial seawater was used (Marine Enterprises International). All experiments were run at salinity 28 except for one study where salinity 11 was used. Electric immersion heaters were used to maintain hyperthermic conditions at 32°C, while room temperature (RT) tanks varied between 25 and 28°C. The water temperature was monitored twice daily with a hand-held thermometer. Shrimp were fed pelleted

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Rangen feed (Buhl, ID; 35% protein) ad libitum. The bioassays were performed once and terminated 36 to 72 h following the last mortality in the positive control tanks.

**Bioassay 1: infection with TSV-BZ via 2 routes of exposure at 32°C.** The set up of the experiment for both routes of exposure was as follows: 2 exposed tanks at 32°C, 1 exposed tank at RT and 1 non-exposed tank at 32°C. The shrimp density was 10 per tank (11 shrimp for one of the TSV-injected hyperthermic tanks). Shrimp exposed by injection received 50 µl in the second abdominal segment of a 1:10 dilution in 2% saline of TSV-BZ infected tissue homogenate. The negative control shrimp were injected with 50 µl of SPF tissue homogenate diluted 1:10 in 2% saline. The shrimp infected by the oral route were fed 10% of their body weight (BW) of TSV-BZ infected minced tissues on Days 1 and 2. Negative control shrimp for the feeding experiment were fed Rangen feed only.

Throughout the assay, moribund shrimp were fixed with Davidson's AFA for examination by histopathology (Bell & Lightner 1988). The pleopods of these shrimp were fixed in 95% ethanol prior to RNA extraction with a High Pure RNA Tissue kit (Roche) according to the manufacturer's recommendation (standard reaction). The virus was quantified by qRT-PCR using a Taq Man probe and normalized to total RNA (Tang et al. 2004). At the end of the assay, survivors were fixed for examination by histology for signs of infection by TSV.

**Bioassay 2: per os infection with TSV-BZ at 32°C and salinity 11.** For this experiment, all tanks were hyperthermic with 2 TSV-exposed tanks at 11 ppt (n = 11 each), 1 TSV-exposed tank at 28 ppt (n = 10) and 1 non-exposed tank at 11 ppt (n = 10). The salinity of the water was measured twice daily with a hand-held refractometer. At the end of the assay, survivors were examined by histology for signs of infection by TSV.

**Bioassay 3: infection with TSV-HI at 32°C.** The design of this experiment was 2 TSV-exposed tanks at 32°C (n = 10 each), 1 TSV-exposed tank at RT (n = 9) and 1 non-exposed tank at 32°C (n = 9). The injection was performed as for Bioassay 1. During the assay, 1 moribund shrimp from the RT TSV-exposed tank was fixed for histopathology. Two survivors per tank were fixed and processed for histopathological analysis and *in situ* hybridization (ISH) (Mari et al. 1998).

**Bioassay 4: time-course of the viral load with infection with TSV-HI at 32°C.** This experiment consisted of 2 treatment tanks at RT and 2 treatment tanks at 32°C. The shrimp density in each treatment tank was 25. Two negative control tanks, each with 5 shrimp injected with 2% saline, were kept at both RT and high

temperature. At Time 0, the treatment shrimp were injected with TSV-HI. Each exposed tank was sampled (n = 4 to 5) every 12 h post-infection (pi), with 9 shrimp sampled at 12, 24 and 36 h for the 32°C treatment, 10 shrimp at 12, 24 and 36 h for the RT treatment and 10 shrimp for both the 32°C and RT treatment at the 48 h time point. At the selected time-points, shrimp were randomly selected and killed. Their pleopods were excised and preserved in ethanol until used for RNA extraction and Real-time qRT-PCR. Histology was performed on 5 shrimp per infected tank (10 shrimp per treatment) and 2 shrimp per negative control tank collected at 48 h pi.

## RESULTS

### Bioassay 1: infection with TSV-BZ via 2 routes of exposure at 32°C

During the assay, 5 hyperthermic TSV-injected, 1 RT TSV-injected, 4 hyperthermic TSV-fed and 2 RT TSV-fed shrimp were sampled. Shrimp presented low survival when challenged with TSV-BZ by either per os (20%) (data not shown) or injection-mediated (21%) exposure at 32°C (Fig. 1). Negative control shrimp kept at this temperature did not present any mortality and had no lesions indicative of TSV by histology. All moribund shrimp collected throughout this assay presented lesions characteristic of the acute or transitional phase of TSV. In these shrimp, viral copy number, varied from  $1.87 \times 10^6$  to  $2.78 \times 10^8$ . All TSV-exposed survivors had severe lymphoid organ spheroids (LOS) suggestive of a chronic TSV infection (Hasson et al. 1995). No other signs of pathology were found in these shrimp.

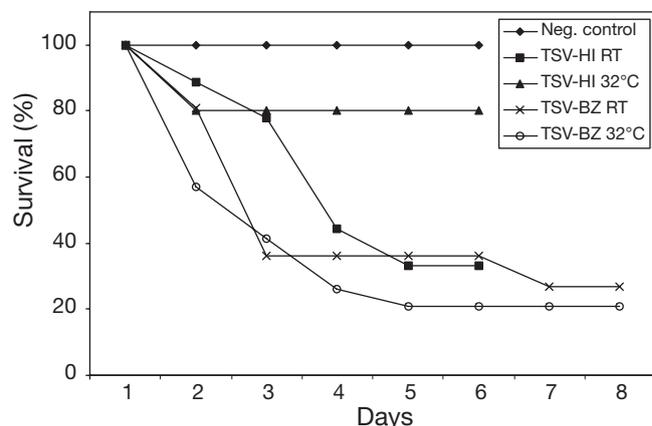


Fig. 1. *Penaeus vannamei*. Survival of shrimp injected with TSV-BZ or TSV-HI under hyperthermic conditions or at room temperature (RT, 25 to 28°C)

### Bioassay 2: per os infection with TSV-BZ at 32°C and salinity 11

During the challenge 9 moribund shrimp from the low salinity tanks were fixed for examination by histopathology. Only dead autolyzed shrimp unsuitable for histologic analysis were found in the high salinity exposed tank. The average survival in the 2 TSV-BZ infected tanks at salinity 11 was 9%. The tank at high salinity (28) had a survival of 30%. All moribund shrimp sampled throughout the bioassay had lesions suggestive of acute or transitional TSV infection. All exposed survivors sampled at the end of the bioassay presented severe LOS suggestive of a chronic TSV infection. Non-exposed shrimp were normal upon histological examination and had 100% survival.

### Bioassay 3: infection with TSV-HI at 32°C

Shrimp exposed to TSV-HI at 32°C presented higher survival (80%) compared with exposed shrimp kept at RT (33% survival) (Fig. 1). Upon histological examination, 1 shrimp from the positive control group fixed 3 d after the start of the challenge had lesions typical of acute TSV infection. The other exposed shrimp from this assay were found dead and were unsuitable for histological analysis. LOS were observed in the 2 survivors from the positive control and at a low to moderate grade in 2 out of 4 examined shrimp from the tanks at 32°C. The LOS from infected shrimp kept at high temperature were poorly organized and negative for TSV by ISH.

### Bioassay 4: time-course of the viral load with infection with TSV-HI at 32°C

There was a large variation in viral copy number  $\text{ng}^{-1}$  RNA amongst shrimp within each treatment (Table 1). Viral copies in the low temperature treat-

Table 1. *Penaeus vannamei*. Lesions, lymphoid organ spheroids (LOS) and maximal viral loads as determined by qRT-PCR following exposure by injection to TSV-HI or TSV-BZ at 2 temperatures. Shrimp challenged with the TSV-HI genotype at 32°C presented 10000× fewer viral copies than did shrimp challenged at room temperature (RT) (25 to 28°C)

Isolate	Acute lesions		LOS		Viral load (max.)	
	RT	32°C	RT	32°C	RT	32°C
TSV-BZ	+	+	+	+	$10^8$	$10^8$
TSV-HI	+	-	+	* <sup>a</sup>	$10^6$	$10^2$

<sup>a</sup>Atypical LOS

ments were consistently higher compared to the high temperature treatments. Shrimp exposed at 32°C presented low titers that decreased following challenge. TSV was not detected by real-time qRT-PCR in shrimp from the negative control tanks. Histopathology results from TSV-HI challenged shrimp showed low levels of early acute phase TSV lesions in 6 out of 10 shrimp under low temperature conditions and none of the shrimp under hyperthermic conditions (number examined = 10).

## DISCUSSION

Shrimp exposed to TSV-BZ presented high mortality at 32°C, independent of the route of exposure (per os or injection) and the salinity of the water (11 vs. 28 ppt). TSV-BZ infected shrimp had severe lesions typical of TSV associated with high viral copy number that indicate the replication of the virus at the higher temperature.

Shrimp challenged with TSV-HI at 32°C presented lower mortalities compared to shrimp kept at RT. The hyperthermic shrimp did not show sign of TSV-HI infection throughout the challenge. They were also free of typical TSV lesions by histopathology and presented very low viral copy number. This confirms that shrimp are protected against TSV-HI infection at 32°C under the conditions examined. This infection pattern is consistent with the behavior of other shrimp viruses. Hyperthermia has been shown to reduce shrimp susceptibility to viral infections such as white spot syndrome virus (Vidal et al. 2001, Granja et al. 2003) and infectious hypodermal and hematopoietic virus (D. Montgomery-Brock et al. 2007).

TSV-BZ might be a temperature-permissible mutant of TSV. Temperature-sensitive mutants, sometimes varying in as little as one nucleotide, are known to have different permissible temperatures. A single-nucleotide change in a capsid protein of poliovirus was sufficient to prevent the maturation of the virus at the non-permissible higher temperature (Compton et al. 1990). Temperature-sensitive RNA polymerase mutants of the foot-and-mouth disease virus (FMDV, a picornaviridae) have been described (Lowe et al. 1981). The mutants were the result of a single-base-pair change in the gene encoding the RNA polymerase. The partial blockage of FMDV at higher temperature has long been recognized (Sharpe 1958).

In view of these results, we conclude that infection with TSV-BZ causes mortality under hyperthermic (32°C) conditions in Kona stock *Penaeus vannamei*, independent of the route of exposure and the salinity (11 and 28 ppt), and that TSV-BZ may be a temperature-permissible mutant of TSV.

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