White spot syndrome virus (WSSV) allows acute hepatopancreatic necrosis disease (AHPND) to cause faster and higher mortality in Pacific white shrimp (*Penaeus vannamei*)

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Introduction

Under the field conditions, multiple infections by more than one pathogen can cause much greater losses in shrimp culture than any single infection. White spot syndrome virus (WSSV) and acute hepatopancreatic necrosis disease (AHPND) infects shrimps and spread rapidly causing a serious loss of the shrimp production in the world. We hypothesized that WSSV infection without clinical symptoms would allow AHPND to cause faster and higher mortality of pond cultured shrimp than those infected with AHPND alone. In this study, co-infection of WSSV and AHPND-causing *V. parahaemolyticus* (Vp_{AHPND}), was studied in juvenile *Penaeus vannamei* under the laboratory condition.

Materials & methods

❖ Experimental design for WSSV and Vp_{AHPND} co-infection

The experimental shrimp (average 0.5g, *N*=80) were divided into 4 groups of 20 shrimp each. The shrimp in each group were sub-divided into 4 replicates of 5 shrimp each in different 6L tanks. Two days after WSSV infection, shrimp were challenged with AHPND and representative shrimp were randomly selected and examined for both WSSV and Vp_{AHPND}, by qPCR, IHC and histopathology.

Group 1: WSSV only	**Group 2: Vp_{AHPND} only**
Group 3: WSSV+ Vp_{AHPND}	**Group 4: not challenged**

Results

Table 1. WSSV and AHPND detection by the qPCR assay (the TaqMan real-time PCR). Shrimp in group 1 were infected with WSSV only (low dose) and WSSV was not detectible by qPCR. However, shrimp in group 3 were infected with WSSV and AHPND both, showing strong WSSV positive by qPCR. This means that secondary infection (AHPND) can trigger WSSV.

Groups	Pathogens	WSSV (Ct values)	AHPND (Ct values)
C1	WSSV	Negative	ND [a]
C2	AHPND	ND	17.68±0.28
C3	WSSV + AHPND	31.76±0.50	17.34±0.22
C4	-	Negative	Negative

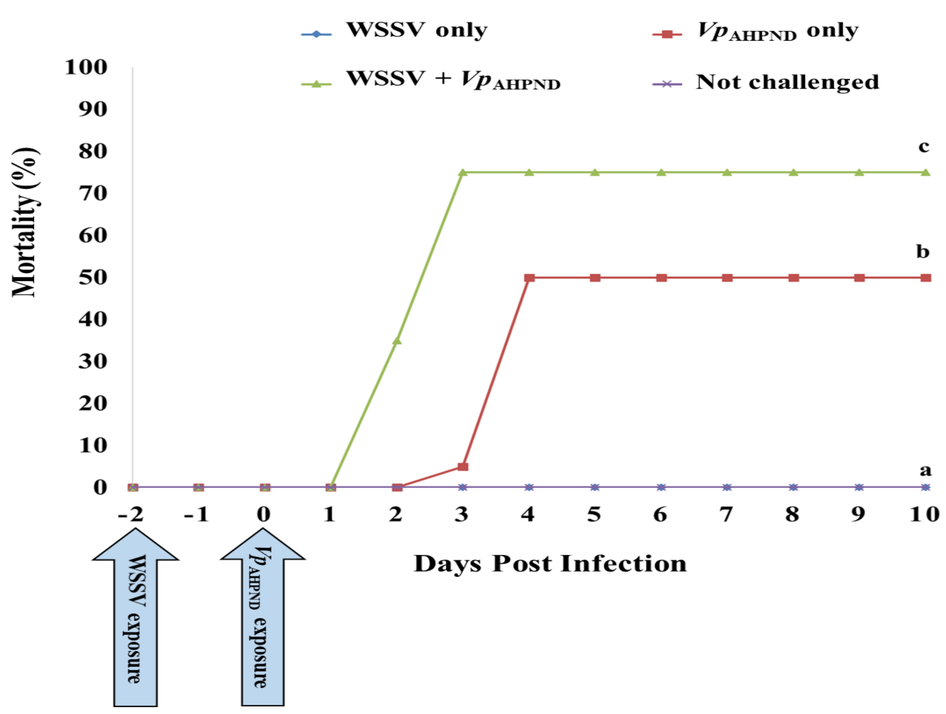


Figure 1. Accumulative mortality of *P. vannamei* after challenge with WSSV and Vp_{AHPND} (group 1: WSSV only; group 2: Vp_{AHPND} only, group 3: WSSV + Vp_{AHPND}; group 4: not challenged). Shrimp mortality was accelerated in the co-infection (WSSV and AHPND), compared to the single infection (either WSSV or AHPND only).

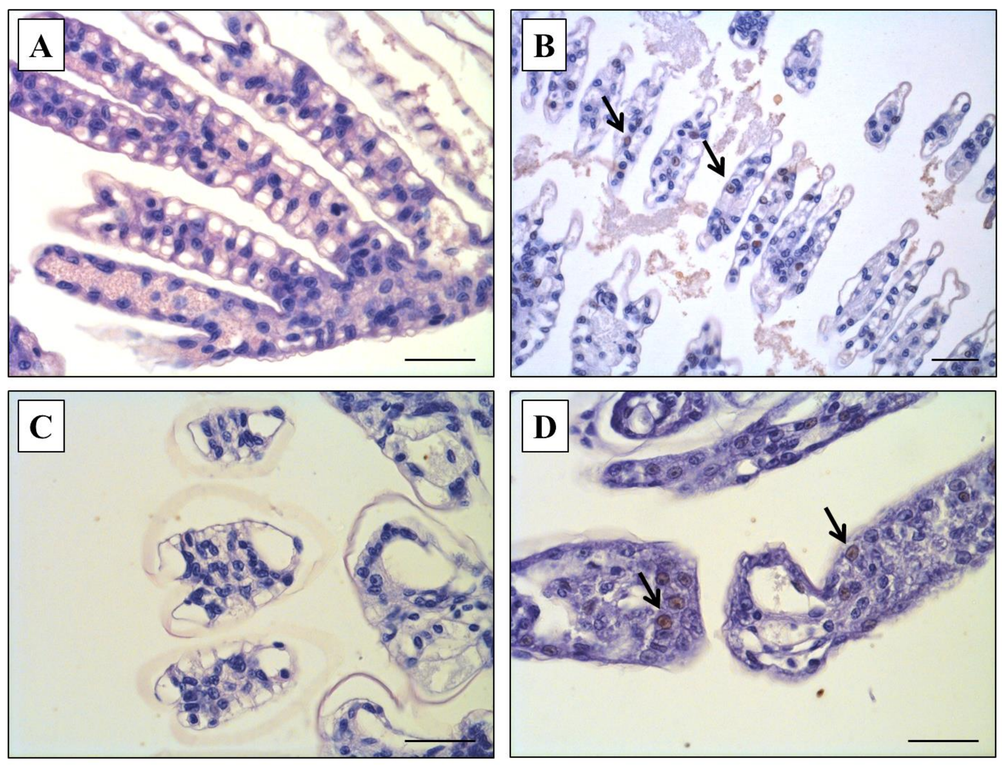
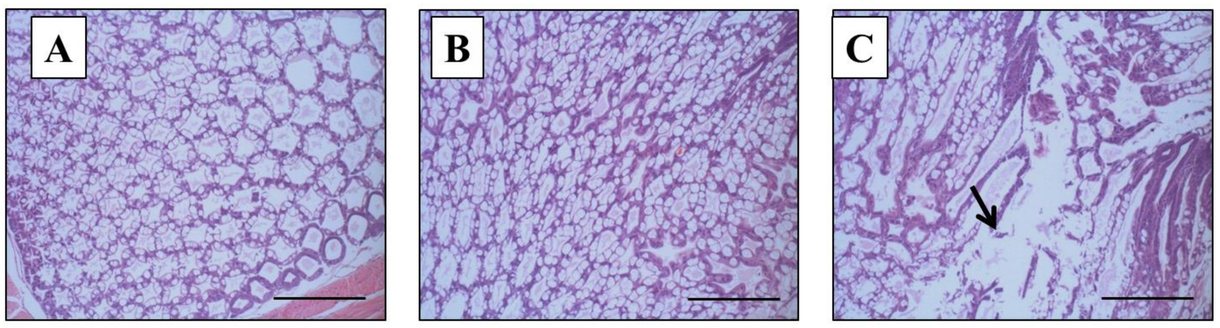


Figure 2. Immunohistochemistry (IHC) examination for WSSV confirmation. (A) NC (group C4), (B) PC (from the preliminary study by feeding method), (C) WSSV control (group C1), (D) WSSV and AHPND co-infection (group C3). Scale bar: 40 μm. WSSV was negative in the shrimp in group 1, but positive in the shrimp in group 3. This means that secondary infection (AHPND) can trigger WSSV in shrimp.



Figrue 3. Histopathology examination for AHPND confirmation. (A) NC (group 4), (B) AHPND control (group 2), (C) WSSV and AHPND co-infection (group 3). H&E staining. Scale bars = 180 μm. AHPND infection was severe in the shrimp in group 3 (WSSV and AHPND), than the shrimp in group 1 (AHPND only).

Conclusions

- This is the first report examining the co-infection of WSSV and AHPND-*Vibrio parahaemolyticus* in juvenile *P. vannamei* under laboratory conditions.
- First, the acceleration in the mortality of shrimp (25% higher cumulative mortality than the AHPND single infection) was revealed by co-infection with WSSV and AHPND.
- Also, the level of WSSV infection was severely increased after the secondary infection with AHPND, confirmed by the immunohistochemistry examination and the qPCR assay.
- Additionally, shrimp initially exposed to WSSV without symptom or mortality were hardly recovered from the AHPND, confirmed by the histopathology examination.
- To sum up, these findings demonstrate that increased susceptibility of WSSV infected shrimp to AHPND, indicating that WSSV infection is a risk factor for AHPND in shrimp ponds.

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (NRF-2019R1C1C1006212 and NRF-2018R1C1B5086350).