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Health &amp; Welfare

# Necrosis due to WSSV can mimic YHV lesions in lymphoid organ of penaeid shrimp

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## Get confirmation by *in situ* hybridization and/or RT-PCR

During the past five or six years, several reports on the presence of Yellow Head Virus (YHV) originated from the United States and other countries in the Americas. In some of the early reports, diagnosis of the disease was based on histological analysis and the demonstration of lesions considered to be “typical.”

## YHV and lymphoid necrosis

Severe necrosis (localized death of cells or tissues through injury or disease) of the lymphoid organ is perhaps one of the most conspicuous and “typical” lesions caused by YHV. Shrimp with acute YHV infection display a generalized multifocal to diffuse severe necrosis, with prominent nuclear pyknosis (condensation of nuclear material) and karyorrhexis (degenerative cellular fragmentation), accompanied by extensive degenerative changes of this organ. This necrosis destroys the parenchymal cells (sheath cells) and fibrous connective tissue cells of the lymphoid organ. Advanced necrosis results in an increased number of blood cells and an abundance of necrotic debris, with the remnants of fragmented nuclei giving a peppered appearance to the lymphoid organ. For several years after YHV was first discovered, necrosis of this type resulted in diagnosis of YHV disease.

## WSSV and lymphoid necrosis

White Spot Syndrome Virus (WSSV) and YHV infect the same tissues and organs of ectodermal and mesodermal origin, including the lymphoid organ. The ectoderm is the outer germ layer, while the mesoderm is the middle germ layer in an embryo.

In some instances, penaeid shrimp with severe WSSV infection also exhibit marked lymphoid organ necrosis almost identical to that caused by YHV. One of the differences is that with WSSV infection, characteristic intranuclear inclusion bodies can be observed in the sheath cells and supporting connective tissue of the lymphoid organ. Such intranuclear inclusions do not occur when only YHV is the infecting agent.

## Dual infection

Since dual infection by WSSV and YHV is possible, the question arises as to whether the severe lymphoid organ necrosis observed in some severe WSSV infections can be the result of YHV. To test this hypothesis, eight representative specimens of WSSV-infected shrimp with severe lymphoid necrosis (determined by H&E histology) were selected from archival material and cases submitted for a second opinion to the Department of Veterinary Science and Microbiology at the University of Arizona in Tucson, Arizona, USA.

The submitted specimens originated from several countries including Mexico, Honduras, and Ecuador, while other specimens were from experimental infections in which only WSSV was used as the inoculum. In each instance, three consecutive sections from each specimen were subjected to H&E conventional histology, *in situ* hybridization with the YHV gene probe, and *in situ* hybridization with the WSSV gene probes, respectively.

## *In situ* hybridization results

No reaction to the YHV probe was observed within the lymphoid organ, or in any other tissue, of the eight specimens tested. In contrast, a positive reaction to the WSSV probes was observed in all of the shrimp examined (Table 1).

## Pantoja, Summary of *in situ* hybridization results, Table 1

Test ID#	Species	Origin	WSSV	YHV	Other
96-183	<i>P. setiferus</i>	Bioassay	+ (LO)	NR	–
99-017	<i>P. stylirostris</i>	Honduras	+ (LO)	NR	TSV + (LO)
99-307	<i>P. vannamei</i>	Honduras	+ (LO)	NR	–
99-326	<i>P. stylirostris</i>	Mexico	+ (LO)	NR	TSV + (LO)
00-058	<i>P. vannamei</i>	Bioassay	+ (LO)	NR	–
00-204	<i>P. vannamei</i>	Ecuador	+ (LO)	NR	–
00-294	<i>P. stylirostris</i>	Honduras	+ (LO)	NR	–
00-359	<i>P. stylirostris</i>	Mexico	+ (LO)	NR	–

WSSV = White Spot Syndrome Virus LO = Lymphoid organ

YHV = Yellow Head Virus NR = No reaction

TSV = Taura Syndrome Virus

Table 1. Summary of *in situ* hybridization results.

Two of the eight specimens examined also had been previously subjected to *in situ* hybridization with the gene probe for detection of Taura Syndrome Virus (TSV), and a positive reaction was observed within the lymphoid organ. This reaction to the TSV probe confirmed the integrity of the viral RNA and also served as an internal control for the validity of the test in detecting possible infection by other RNA viruses.

## Conclusion

Since this experiment demonstrated that severe lymphoid organ necrosis can also appear as a result of severe WSSV infection, it is recommended not to use this type of lesion as the sole feature to diagnose YHV disease. In cases where YHV infection is suspected after conventional H&E analysis, additional confirmation by other means – such as *in situ* hybridization and/or RT-PCR – should be carried out.

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