K-law spectral signature correlation algorithm to identify white spot syndrome virus in shrimp tissues

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ABSTRACT

An algorithm is developed to identify the white spot syndrome virus (WSSV) inclusion bodies, found in shrimp tissues by the analysis of digitalized images from infected samples. WSSV slide images were acquired by a computational image capture system and a new identification algorithm is developed to obtain those infected shrimp samples by the quantitative measurement of the complexity pattern found in WSSV inclusion bodies. Representative groups of WSSV inclusion bodies from infected shrimp tissues and organs were analyzed.

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1. Introduction

Since 1992 to date, have been reported near to twenty viruses to infect marine shrimps, however just seven of these viral pathogens are currently mentioned by the World Organization for Animal Health (OIE) as causing important losses in the shrimps population (OIE, 2010). White spot syndrome (WSSV) is by far the most devastating pathogen of the farmed shrimp, affecting the economy of shrimp producers around the world restraining aquaculture production (Walker and Mohan, 2009). White spot syndrome disease is an infection caused by the virus named WSSV, which is the only member of the *Nimaviridae* family. The virions have a double strain of DNA, are ovoid, ellipsoid or bacilliform in shape, have a trilaminar membrane and measure 120–150×270–290 nm in size. The genome size is approximately 290 kbp. The epidemics is characterized by a rapid and increase mortality showing symptoms of anorexia, lethargy, in some cases Asian species shows the presence of white spots in the cephalotorax as characteristic of the disease, while in the American species of penaeids, infected or moribund shrimp have reddish coloration due to the expansion of chromatophore (Lightner, 1996; Lightner and Pantoja, 2001; OIE, 2010). WSSV can spread and infect shrimps of any stage of grow-out, asymptomatically affecting all life cycle stages, from eggs to broodstock. Once the clinical signs are developed, mortality can reach 100% in 3 days. WSSV is a highly contagious viral disease of penaeid prawns (Penaeidae family), However, all decapod crustaceans including prawns, lobsters and crabs from marine, brackish water or fresh water are considered susceptible to the infection (OIE, 2010).

In México, several shrimp producers from Sonora, Sinaloa and Nayarit states reflected their losses by the reduction of exportations from 30 million USD in 2000 compared to the 45 million USD in 1999, the losses amount were approximately 15 million USD just in one production year. After this year producers, authorities and academy have been taking actions to control the WSSV disease to reduce the impact, however still is causing important losses. Sinaloa has lost by the impact of this virus the amount of $5,059,956.000 from 2003 to 2009 (Aquatic Health Committee of Sinaloa State (CESASIN), 2010, personal communication). The state of Sonora has lost by the same pathogen a total of $1,800,000,000 in 2004 to 2010 (Aquatic Health Committee of Sonora State (COSAES), 2010, personal communication). Baja California Sur State lost during one outbreak of WSSV in 2008, the amount of 30 tons so entailed a loss of about $1,500,000 (Aquatic Health Committee of Baja California Sur (CESABCS), 2010, personal communication). Information is not available from Nayarit State, but there is no doubt that the effects had also similar impact.

Several techniques have been implemented and developed for viral and bacterial penaeid shrimps diagnostics; these can be divided in traditional morphological pathology, bioassay, microbiology, molecular methods such as polymerase chain reaction (PCR) and implementation

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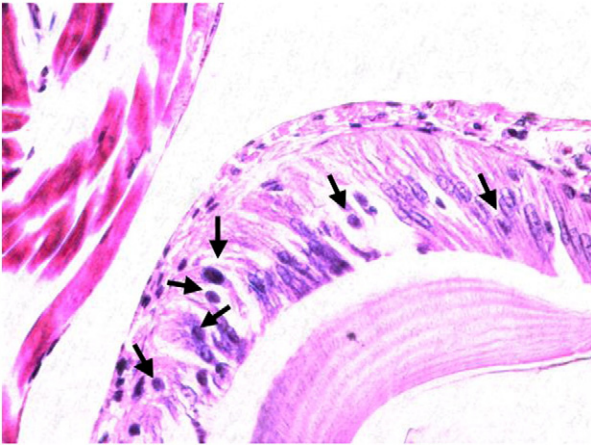
Fig. 1. Arrows show the effects of WSSV infection on cuticular epithelium cells from a histological shrimp tissue sample.

of monoclonal antibodies (Mabs) for the detection of WSSV in slices of paraffin (Lightner, 1996; OIE, 2010), however three methods are used traditionally for WSSV diagnostics: histological analysis, in situ hybridization on fixed tissues with WSSV specific gene probes and PCR method with specific oligonucleotide primers (Poulos et al., 2001).

Histology is still considered the common tool in medical and veterinary for research and diagnostics tasks (Lightner and Redman, 1998). Sometimes for massive diagnostic requirements or epidemiological studies requires a considerable amount of slides that have to be analyzed to determine pathological changes in several tissue cells or to allow the pathogen identification which are sometimes difficult to recognize with other alternative techniques. For this kind of analysis the method involves several steps to obtain the final sample, which is a tissue slice of 5 μm thickness, stained with hematoxilin–eosin necessarily to make the examination under microscope (Bell and Lightner, 1988; Lightner, 1996; Lightner and Redman, 1998), as shown in Fig. 1.

WSSV infection is commonly seen in cuticular epithelial cells and connective tissue cells of the stomach and gills. However it is also seen in antennal gland, lymphoid organ, hematopoietic tissue and phagocytes of the heart. Infected cells typically have hypertrophied (enlarged) nuclei containing a single intranuclear inclusion. Inclusions at the beginning are eosinophilic and sometimes are separated by a clear halo beneath the nuclear membrane; these are known as Cowdry type A inclusions. Later inclusions become lightly to deeply basophilic and fill the entire nucleus (Lightner and Pantoja, 2001; OIE, 2010), as shown in Fig. 2.
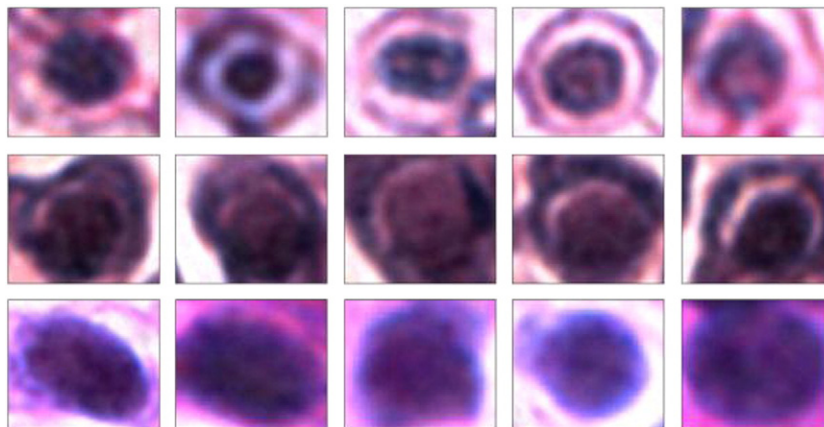
The need for rapid, sensitive diagnostic methods led to develop new alternative techniques in different fields of knowledge like computing optic disciplines, which can be of support to conventional methods. Several optic and computational techniques were developed to recognize these kinds of biological patterns, the analysis of inclusion bodies is determinant of the virus presence, thus color correlation approach was used to analyze and recognize the presence of Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) inclusion bodies by histological samples from 35 mm transparencies digitalized with a flatbed scanner (Álvarez-Borrego and Chávez-Sánchez, 2001) as well as WSSV color images are obtained and analyzed in this paper.

The aim of this manuscript is to show a new computer algorithm capable to analyze several shrimp tissue samples infected by WSSV basophilic and Cowdry type A inclusion bodies acquired from histological digitalized color images, by applying Fourier spectral filtering techniques over these slide samples, such as K-Law nonlinear filter.

These Fourier spectral and color correlation techniques have demonstrated the capability to analyze important characteristics from viruses and pathogens (Álvarez-Borrego and Chávez-Sánchez, 2001; Mouriño-Pérez et al., 2006), including applications in several fields (Coronel-Beltrán and Álvarez-Borrego, 2010; Millán et al., 1992).

2. Materials and methods

2.1. Virus sample preparation

Experimental shrimps were obtained from a farm located in the state of Sinaloa, México; transported alive to the laboratory to be fixed in Davidson's solution; after 24 h, the fixative was discarded and shrimps were preserved in 50% alcohol solution until they were ready to be processed by conventional histology techniques, as suggested by Lightner (1996) and Lightner and Redman (1998).

Once histological slides were prepared and ready to be examined under microscope, different types of WSSV inclusion bodies were selected from cuticular epithelium, connective tissue and abdomen tissue, afterwards multispectral digitalized images were obtained to construct a comparison inclusion bodies filter bank; subsequently several images were acquired from the shrimp's slide samples to be diagnosed by KSCA, like WSSV shrimp's infected tissue image, as shown in Fig. 1.

2.2. Digitalized images capture

The WSSV slide images were acquired by a computational system of capture images as shown in Fig. 3, including proprietary image processing software, to enhance the digitalized images with novel

Fig. 2. WSSV basophilic and Cowdry type A inclusion bodies.