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ORIGINAL ARTICLE

The importance of histopathological diagnosis in assessing the impact on the prognosis of White Spot Disease in cultures of Pacific white shrimp (*Penaeus vannamei*)

Pedro Anderson de Paiva dos Santos[1], Luana Bortolini Giesta[1*], Fernando Pablo Silva Oliveira[1], Virgínia Fonseca Pedrosa[2], Luis Alberto Romano[1]

Abstract - Aquaculture is a globally relevant sector in terms of production and income. When we think about shrimp farming, *Penaeus vannamei*, the Pacific white shrimp, has been instrumental in this growth due to its characteristics and advances in production. Despite achievements, it faces significant economic challenges due to the white spot syndrome virus (WSSV), causing considerable losses. This virus has led to significant economic losses, affecting economies such as Ecuador's. The clinical signs of WSSV require rigorous diagnostic procedures, combining histopathological examination and PCR assays. A retrospective study of 900 specimens of *Penaeus vannamei* in Ecuador highlights the correlation between tissue pathology and mortality rates, enhancing understanding of the direct impact of the disease. Histopathological analysis reveals distinctive intracellular viral inclusions, reflecting disease progression. Although PCR assays provide definitive diagnoses, histopathological evaluation enriches understanding of lesion severity, supporting a comprehensive approach to disease management strategies.

Keywords: Histopathology. Shrimp. Virus.

A importância do diagnóstico histopatológico na avaliação do impacto no prognóstico da doença da mancha branca em cultivos de camarão branco do Pacífico (*Penaeus vannamei*)

Resumo - A aquicultura, com ênfase na criação de camarões, tem alcançado considerável produção e receita globalmente. O camarão branco do Pacífico, *Penaeus vannamei*, desempenha um papel fundamental nesse crescimento, devido às suas características e aos avanços nos processos de cultivo. Apesar desses avanços, desafios econômicos significativos persistem devido a invasões patogênicas, especialmente a síndrome da mancha branca (WSSV), resultando em mortalidades consideráveis. O WSSV, um agente patogênico viral, causou perdas financeiras bilionárias globalmente, afetando economias como a do Equador. Os sinais clínicos do WSSV, incluindo anorexia e letargia, exigem procedimentos diagnósticos rigorosos, combinando exame histopatológico e ensaios de PCR. Este estudo investigou retrospectivamente 900 espécimes de *Penaeus vannamei* no Equador, destacando a correlação entre patologia tecidual e taxas de mortalidade, elucidando o impacto direto da doença. O exame histopatológico revelou inclusões virais intracelulares distintas em vários tecidos, refletindo a progressão da doença. Embora os ensaios de PCR forneçam diagnósticos definitivos, a análise histopatológica complementa a compreensão da gravidade das lesões, promovendo uma abordagem diagnóstica abrangente nas estratégias de manejo da doença.

Palavras-chave: Histopatologia. Camarão. Vírus.

[1] Universidade Federal do Rio Grande (FURG) – Laboratório de Imunologia e Patologia de Organismos Aquáticos (LIPOA). *Corresponding author: luanabortolinigiesta@gmail.com.
[2] Universidade Federal de Alagoas (UFAL) - Laboratório de aquicultura e ecologia aquática (LAQUA).

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Introduction

Aquaculture plays an indispensable role in the global provision of animal protein. In 2020, shrimp farming, with a production of 11.2 million tons and generating a revenue of $85.5 billion worldwide, emerged as one of the most rapidly expanding sectors within aquaculture (FAO, 2022). This growth has been facilitated mainly by the widespread adoption of *Penaeus vannamei*, commonly known as the Pacific white shrimp, owing to its favorable biological attributes and established technological practices (Natori *et al.*, 2011). However, despite the sector's advancement, shrimp farming encounters significant economic setbacks attributed to the presence of pathogens, including viruses, fungi, bacteria, and parasites, which contribute to mortality and impede further production escalation (Zorriehzahra; Banaederakhshan, 2015).

Viruses and bacteria are the primary pathogens responsible for high mortality rates in crustaceans. Several of these diseases, which require mandatory reporting to the World Organization for Animal Health (OIE), engender substantial losses within the productive sector and escalate mortality rates among farmed animals (Tandel *et al.*, 2017; Flegel, 2019).

Viral infections pose the most formidable challenge in shrimp production, with notable diseases including Infectious Hypodermal and Hematopoietic Necrosis (IHHNV), Taura Syndrome (TSV), Yellow Head Disease (YHV), and White Spot Syndrome Disease (WSSV) (Lee *et al.*, 2022).

Notably, WSSV ranks highest regarding both productive and economic losses among pandemic and endemic diseases globally (Walker; Mohan, 2009; Flegel, 2012; Zou; Liu, 2020).

Clinical signs of WSSV infection include loss of appetite, lethargy, and the development of white spots on the exoskeleton due to calcium deposition. However, white spots alone are not conclusive evidence of WSSV infection since some bacterial diseases cause similar spots. Therefore, histopathological analyses and PCR testing are imperative (Santos *et al.*, 2013). Diagnostic methods for WSSV encompass a range of approaches, with molecular techniques serving as confirmatory measures for sanitary certification mandated by the OIE, with polymerase chain reaction (PCR) emerging as the predominant method (OIE, 2023). Histopathological investigation assumes critical importance in gauging disease prognosis within a population.

In this study, we conducted a retrospective study involving 900 *Penaeus vannamei* specimens collected in Ecuador between November 2001 and November 2003, sourced from shrimp farms in the Guayas and Del Oro provinces, with disease presence validated and certified through PCR.

All specimens underwent histological processing. Subsequently, a comprehensive review of tissue samples from the samples were conducted to evaluate the extent of tissue and organ involvement, aiming to correlate these findings with variations in mortality rates. Our observations confirm a direct relationship between mortality rates and the severity and distribution of WSSV viral particles across different tissues.

Material and Methods

This study involved 900 (300 per farm) *Penaeus vannamei* specimens from aquaculture facilities located in Del Oro Province, with a cultivation area of 124 hectares, and two facilities in Guayas Province, encompassing 57 and 89 hectares, respectively. Sampling was performed across three harvesting cycles at each facility. In adherence to ethical guidelines, the identities of the establishments where the samples were collected are not disclosed.

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Shrimp specimens were collected from the respective facilities upon reaching a moribund state, with the majority displaying characteristic clinical manifestations of the disease. The specimens were fixed using Davidson's fluid injected at multiple points, including laterally at the hepatopancreas level and in the anterior and posterior abdomen regions. Subsequently, the specimens were immersed in the same fixative, following a 3:1 ratio.

Once in the laboratory, all samples underwent processing using an automated tissue processor (LEICA TP1020) and were embedded in paraffin. Subsequently, tissue sections were cut using a semi-automatic microtome (LEICA RM2245) at a thickness of 3 micrometers. For histological examination, the slides were stained with hematoxylin and eosin following the protocol outlined by Luna (1968). The stained slides were then observed under a Zeiss Primo Star optical microscope equipped with an Axiocam ERC5s camera - AxionVision (LE).

Seeding and collection data

Table 1 show seeding data, larval density per hectare, average days per cycle, survival rate, and animal weight at harvest. In all instances, PL 12 larvae were seeded.

Table 1. Seeding data, larval density per hectare, average days per cycle, survival rate, and animal weight at harvest.

Establishment	Planting density	Average age	Average survival	Average weight	Average
	Larvae/ha	Days	%	g	lbs/ha
Del Oro Province	130.924	107.4	14.33	12.02	261.84
Guayas-1 Province	136.76	105.7	37.20	11.12	137.00
Guayas-2 Province	157.30	110.8	65.70	12.60	1432.48

Result and Discussion

The histopathological findings resemble those previously documented in the literature (Wang *et al*., 1999; Hameed *et al.*, 2003; Reddy; Jeyasekaran; Shakila, 2013), with intracellular viral inclusion bodies observed. These intracellular viral inclusion bodies appeared homogeneous and eosinophilic in the early stages of infection, transitioning to a basophilic state in late-stage lesions (Fig. 1).

Such cellular alterations were evident across various tissues and organs, including the stomach, gills, epidermis, hepatopancreas, heart, lymphoid organ, hematopoietic tissue, antennal gland, intestine, nervous tissue, and ocular tissues. Predominantly, alterations were noted in the epidermis, gastric epithelium, and gills (Fig. 2, 3, 4 and 5).

The extent of lesions in tissues, was assessed in the organs exhibiting cellular lesions due to viral invasion. Table 2 illustrates the relationship between mortality rates and the percentage of affected organs. It’s possible to understand the correlation between mortality rates and tissue involvement

The PCR is a practical tool for reliable diagnoses with high effectiveness, indicating the presence or absence of viral genetic material (Nunan; Lightner, 1997). The classical and molecular histopathology allows to evaluate the severity, extent, and pathogenesis of lesions. Thus, histopathological diagnosis and PCR complement each other.

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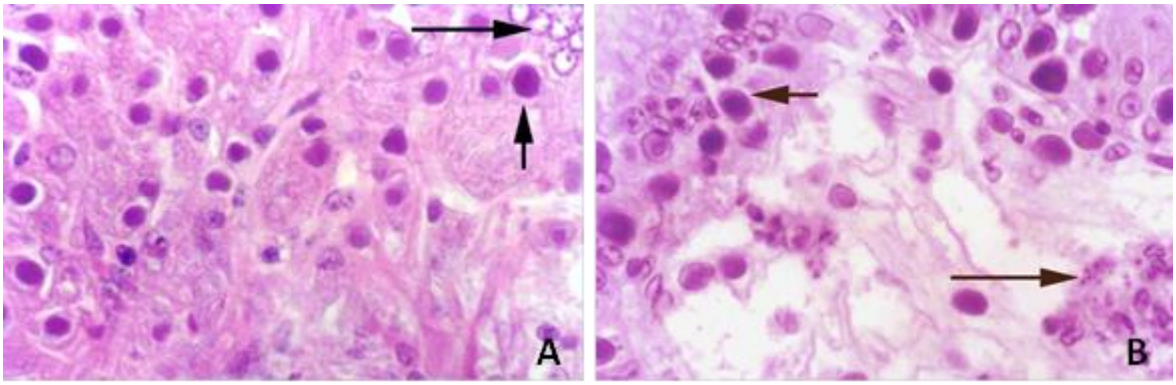

Figure 1: A. Eosinophilic nuclear inclusions can be observed (short arrow), and in some cases, chromatin marginalization can be seen (long arrow). Hematoxylin and eosin staining, magnification of 40 times (H&E X 40). **B.** Basophilic inclusions with homogeneous hyperchromatic nuclei (short arrow), with areas of cellular fragmentation (long arrow). Hematoxylin and eosin staining, magnification of 80 times (H&E 80X).

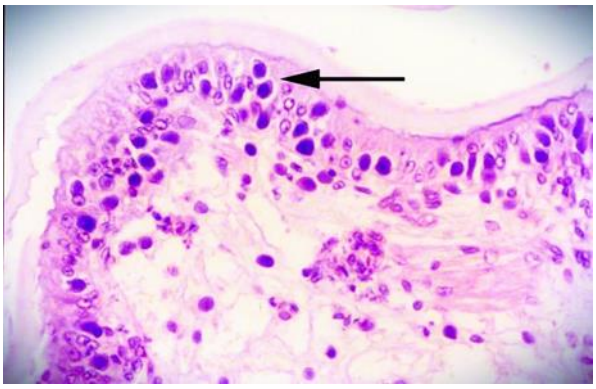

Figure 2. Cuticle and epidermal epithelium with viral inclusions (arrow). Magnification of 40 times (H-H 40X).

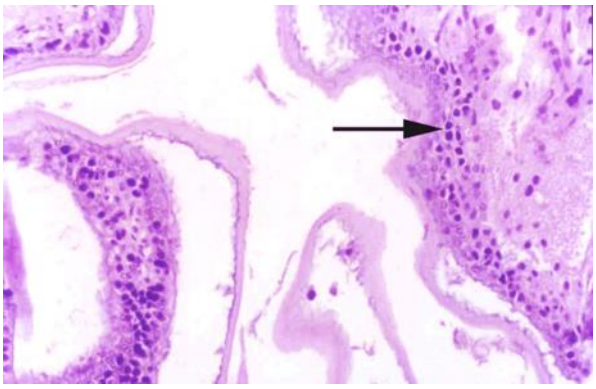

Figure 3. Gastric epithelium with multiple viral inclusions (arrow). Magnification of 40 times (H-H 40X).

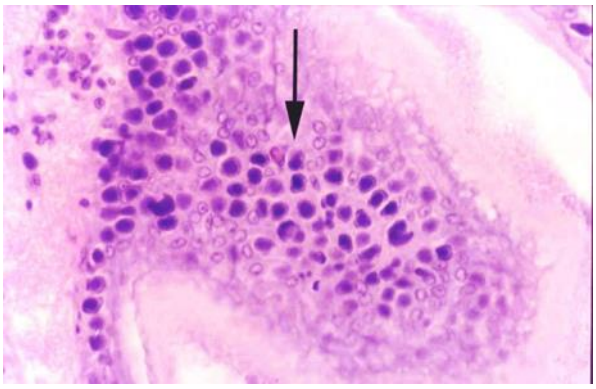

Figure 4. Tangential section of the gastric epithelium with multiple viral inclusions (arrow). Magnification of 60 times (H-H 60X).

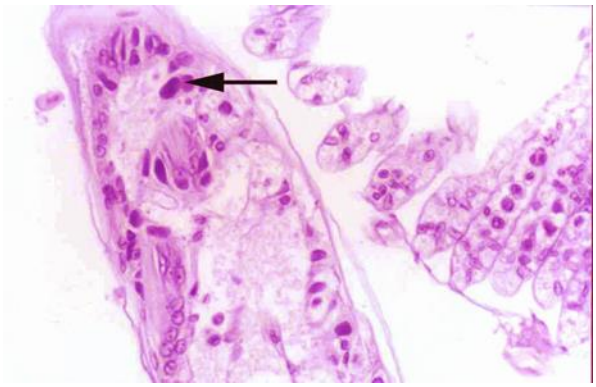

Figure 5. Gill filaments with viral inclusions (arrow). Magnification of 40 times (H-H 40X).

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Table 2. Correlation between mortality rates and tissue involvement.

	Del Oro Province Affected (%)	Guayas-1 Province Affected (%)	Guayas-2 Province Affected (%)
Average mortality	85.6%	63%	34%
Tissue involvement			
Stomach	100	100	100
Epidermis	100	100	100
Gill	83.7	32.7	2.7
Hepatopancreas	55.7	18.6	0
Hearth	12.4	8.3	0
Antennal Gland	9.5	3.8	0
Hematopoietic Tissue	7.4	2.9	0
Intestine	5.4	1.8	0
Ocular Tissue	3.5	0	0
Nervous Tissue	1.4	0	0

The histopathological diagnosis of WSSV relies on demonstrating intranuclear viral inclusion bodies, initially eosinophilic and later becoming basophilic, with dense and homogeneous staining observed with hematoxylin and eosin. Infected cells typically exhibit hyperchromatic nuclei with marginal chromatin, predominantly in the epithelial cells of the stomach and gills. The epidermis, hemocytes, and connective tissue cells can also be affected. In more advanced stages of infection, cellular cytoplasm disintegration can be observed with little or no inflammatory response (Durand; Lightner, 2002; Wang *et al.*, 2002; Escobedo-Bonilla *et al.* 2007). Additionally, viral tropism is evident in various tissues, including the hepatopancreas, lymphoid organs, antennal gland, muscular tissue, pleopods, hematopoietic tissue, heart, intestine, nervous tissue components, eyes, and gonads (Escobedo-Bonilla *et al.* 2007; Zou; Liu, 2020).

A proportional relationship was noted between mortality rates and the number of organs affected by the virus. Specifically, specimens from the Del Oro Province facility, with an average mortality rate of 87.67 %, displayed a higher incidence of affected organs compared to those from the Guayas-2 Province facility, where mortality was below 34 %, with viral involvement confined to the stomach, epidermis, and gills.

It is crucial to recognize that histopathological diagnosis does not supplant molecular techniques but enriches them within a comprehensive diagnostic framework. Each diagnostic method should serve a specific purpose within a protocolized system, ensuring a holistic understanding of diseases such as WSSV. Therefore, the integration of various diagnostic techniques is imperative to gain comprehensive insights into disease pathology.

In conclusion, the histopathological examination of WSSV-infected specimens revealed characteristic cellular alterations consistent with documented findings, highlighting the presence of intracellular viral inclusion bodies across multiple tissues. Our assessment of tissue lesions, mortality rates, and organ involvement underscored a proportional relationship, emphasizing the severity of viral invasion.

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While PCR remains a valuable tool for initial diagnosis, the depth of understanding regarding lesion severity, distribution, and pathogenesis is significantly enhanced through classical and molecular histopathology. Integrating these diagnostic approaches within a structured framework provides a comprehensive understanding of WSSV pathology. Therefore, amalgamating diagnostic methodologies is essential for an effective disease management and control strategies.

Conflict of Interests

The authors declare that the research was conducted in the absence of any potential conflicts of interest.

Ethical Statements

The authors confirm that the ethical guidelines adopted by the journal were followed by this work, and all authors agree with the submission, content and transfer of the publication rights of the article to the journal. They also declare that the work has not been previously published nor is it being considered for publication in another journal.

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ORCID

Pedro Anderson de Paiva dos Santos
https://orcid.org/0009-0003-3201-639X
Luana Bortolini Giesta
https://orcid.org/0009-0007-2189-8357
Fernando Pablo Silva Oliveira
https://orcid.org/0000-0003-2011-8164
Virgínia Fonseca Pedrosa
https://orcid.org/0000-0003-3353-1994h
Luis Alberto Romano
https://orcid.org/0000-0003-3144-3579

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