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Research Article

Absence of inflammatory response by infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Penaeus vannamei* cultivated in northern Peru

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ABSTRACT. Infectious hypodermal and hematopoietic necrosis virus (IHHNV) is a disease of penaeid shrimp caused by decapod *Penstylhamaparvovirus* 1, which infects both wild and cultured *Penaeus vannamei* shrimp and requires mandatory notification to the World Organization Animal Health (WOAH). This report presents the results of the molecular diagnosis of IHHNV and histopathological analysis of cultured *P. vannamei* specimens during the execution of the Pathogens Surveillance Plan (2022) on the Peruvian northern coast. Six epidemiological units located in Tumbes and Piura were sampled. Molecular diagnosis was performed by real-time PCR using a protocol recommended by the WOAH, and histopathological analysis was conducted using the hematoxylin and eosin H&E staining method. Overall, the prevalence of IHHNV-positive cases was 88.66%, with a prevalence greater than 75% per epidemiological unit. After histopathological analysis, all cases exhibited typical or IHHNV-related lesions, including mild nuclear alterations, presence of eosinophilic intranuclear inclusions of Cowdry type A or hypertrophied nuclei in cells of target tissues, such as cuticular, epicardial, and connective tissue. However, the severity of the lesions due to IHHNV infection was low, being categorized as grade 1. No evidence of an inflammatory response was observed, and no mortalities attributed to this pathogen were reported in the sampled epidemiological units. Our results are consistent with recent findings on the presence of endogenous viral elements in the shrimp genome as an adaptive response to IHHNV infections, resulting in no negative impact on *P. vannamei* culture.

Keywords: *Penaeus vannamei*; shrimp culture; infectious hypodermal and hematopoietic necrosis virus (IHHNV); real-time PCR; histopathology; inflammation

INTRODUCTION

Shrimp aquaculture is a rapidly growing economic activity. Its production has increased from 75,000 in 1980 to 5.5 million metric tons in 2017. Even though it has been one of the species most affected by diseases, it continues to be produced on a large scale (Asche et al. 2020). The international movement of crustacean specimens (specifically, commodity shrimp) for the growth and development of the aquaculture penaeid industry has caused the transfer of important pathogens from Asia to America, including the transfer of shrimp viruses such as infectious hypodermal and hematopoietic necrosis virus (IHHNV) were from one continent another, infecting naive hosts with or without native resistance. New diseases have collectively cost billions of dollars (with (IHHNV alone costing US$1 billion), causing a great impact on exports, shrimp farms, and jobs (Lightner 2011). The IHHNV was introduced in America from the Philippines in the 1970s. In 1981, it was discovered in Hawaii, where it caused severe illness in *Penaeus stylirostris*. It was de-

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termined that it could also infect *Penaeus vannamei* and *P. monodon*. Infectious hypodermic and hematopoietic necrosis have been reported in Asia, Oceania, South and North America, the Middle East, East Africa, Australia, and the eastern Indo-Pacific region and is a notifiable disease of penaeid shrimp to the World Organization Animal Health (WOAH) (Lightner 2011, WOAH 2023).

Purified IHHNV particle shows an icosahedral non-enveloped structure of 22 nm in diameter and a single strand (ssDNA) genome estimated at 4.1 kb (Bonami et al. 1990), which contain three open reading frames; two of these frames encode non-structural proteins (NS1 and NS2) while the third encodes the viral capsid protein (CP). The International Committee on Viral Taxonomy (ICTV) has classified it as decapod *Penstylhamaparvovirus* 1, genus *Penstylhammaparvovirus*, subfamily Hammaparvovirinae (Pénzes et al. 2020, Shike et al. 2000).

Parvoviruses can cause acute or persistent infections and integrate their genome into the host chromosome or remain in an episomal state; the immune response mechanism in these cases is unclear (Liu et al. 2011). Recent reports indicate that after IHHNV enters the host cell and the immediate synthesis of viral messenger RNA (mRNA), viral complementary DNA (vcDNA) is then produced by reverse transcription from mRNA using host reverse transcriptase (RT). This synthesis can generate both linear (lvcDNA) and circular (cvcDNA) forms of vcDNA, that induce synthesis of small interfering RNA (siRNA) molecules, resulting in a cellular, systemic, and specific RNA interference (RNAi) response to invading viruses. Some vcDNA enters the nucleus of the host cell and achieve to integrate into the chromosomal DNA, becoming endogenous viral elements (EVE) (Flegel & Pasharawipas 1998, Tang & Lightner 2006, Taengchaiyaphum et al. 2021). EVEs are transcribed into long antisense RNAs, which are then cleaved into small antisense RNAs that specifically interact with P-element induced wimpy testis (PIWI) proteins (piRNAs) to act as a guide in the recognition of positive-sense viral mRNA, triggering its degradation through the RANi pathway. This natural mechanism is believed responsible for balanced persistent infections, where shrimp can tolerate one or more viruses throughout their lives, sometimes without manifesting disease symptoms. This phenomenon of "tolerance" to persistent viral infections is referred to as viral accommodation (Taengchaiyaphum et al. 2021).

IHHNV was reported for the first time on the northern coast of Peru in Tumbes in wild shrimp *P. vannamei* by polymerase chain reaction (PCR) (Alfaro-Aguilera et al. 2010), later in postlarvae of *P. vannamei* introduced to Peru (Guevara & Alfaro 2012). In 2019, the complete genome of IHHNV strains identified in Tumbes-Peru during surveillance of diseases that affect shrimp farming was sequenced (Salcedo-Mejía et al. 2021). Five infectious genotypes of the virus have been described: types A, II, III, and non-infectious genotypes A and B (Shen et al. 2015). Phylogenetic analysis of genomes of strains identified and sequenced in Peru shows that they are grouped in genotype II, where they form a clade separate from strains previously reported in Latin America (Salcedo et al. 2021, Aranguren et al. 2022). Although IHHNV is highly prevalent in shrimp production centers in Latin America, where the presence of the virus has been described in postlarvae, juveniles, and reproducers, no high mortality, clinical signs, or rostrum deformity syndrome (RDS) have been observed. Histopathological findings such as Cowdry type A intranuclear inclusion corpuscles in target tissues confer only a provisional diagnosis of IHHNV infection, characterized by a corpuscle of eosinophilic color, in addition to frequently presenting prominently in the nucleus with a halo around it resulting in cells with hypertrophied nuclei with marginalized chromatin that distorts the visualization of the normal relationship between the nucleus and the cytoplasm (Lightner 1996b, 2011, WOAH 2023). However, the observation of these hypertrophied corpuscles or nuclei with eosinophilic staining are not considered pathognomonic signs of IHHNV disease since it may be a shared feature also observed in the histopathological analysis of early phases or the development of white spot syndrome virus (WSSV). Likewise, the observation of cells of ectodermal and mesodermal origin containing corpuscles or karyo-megaly may indicate IHHNV infection but not a disease caused by this virus, especially in *P. vannamei* cultivated in Ecuador and northern Peru (Aranguren-Caro et al. 2022, WOAH 2023), since it does not represent a major challenge for the immunological response with no or little inflammatory response in the target sites of infection, interpreting it as a low degree of severity that is associated with the current concept of the immunological "tolerance" of crustaceans such as *P. vannamei* for pathogens such as IHHNV (Flegel & Pasharawipas 1998, Lightner & Redman 1998, Flegel 2020, WOAH 2023).

In this article, we present the results of the molecular diagnosis of IHHNV and histopathological analysis of cultured specimens of *P. vannamei*, carried out during the execution of the Epidemiological

Surveillance Plan (2022) on the northern coast of Peru. These findings prove that an IHHNV viral infection does not necessarily result in a notable pathological inflammatory response in shrimp.

MATERIALS AND METHODS

Sample collection

The samples were collected from six epidemiological units distributed on the northern coast of Peru: North 01 (3°27'54.7"S, 80°17'05.4"W), North 02 (3°28'39.4"S, 80°20'16.0"W), Center (3°30'34.0"S, 80°26'41.1"W), South 01 (3°32'44.2"S, 80°30'22.0"W), South 02 (3°48'20.0"S, 80°48'39.3"W), and Piura (5°40'50"S, 80°51'01"W) (Fig. 1). For molecular testing the samples consisted of five shrimp specimens and were submitted to the Organismo Nacional de Sanidad Pesquera (SANIPES) surveillance laboratory maintaining the cold chain. The samples consisted of juvenile shrimp preserved in Davidson solution for histopathological analysis.

Detection of IHHNV by real-time PCR

Following the manufacturer's recommendations, DNA extraction was performed from pleopods using the NucleoSpin® Tissue kit (Macherey-Nagel, Germany). The OIE-recommended method (Tang & Lightner 2001) was used for IHHNV detection. The master mix was performed using the PerfeCTa qPCR ToughMix Low Rox Kit (Quantabio, USA), with the following quantities for a final volume of 20 µL: 10 µL of PerfeCTa qPCR ToughMixLow ROX (2X), 0.6 µL of forward primer and reverse primer (10 µM), 0.2 µL of probe (10 µM), nuclease-free water (up to 23 µL) and 2 µL of template. The PCR cycling program was 95ºC for 3 min (1 cycle), followed by 40 cycles of 95ºC for 15 s and 65ºC for 30 s. Real-time PCR assays were performed using a QuantStudio™ 5 Real-time PCR system - Applied Biosystems™ (Thermo Fisher Scientific Inc.). The B-actin gene was used as an endogenous control using the abovementioned conditions. The primers used are shown in Table 1.

Histopathological analyses

Histopathology of shrimp samples was done following WOAH recommendation. Sixty-four juvenile specimens of *P. vannamei* were collected and preserved by injection and immersion in AFA Davidson fixation solution, alcohol-formalin-acetic acid, according to WOAH recommendations for histopathological analysis (WOAH 2023). Subsequently, tissues corresponding to the cephalothorax and anterior abdominal sections were embedded in wax sectioned between 3 to 5 µm thick using the methodology described by Bell & Lightner (1988). Likewise, light microscopy performed histopathological analysis after staining with the hematoxylin and eosin H&E coloration method. For the histopathological analysis of suspected cases of IHHNV, the degree of severity in tissues from *P. vannamei* was categorized from 0 to 4, as described by Lightner (1996a), where degree 0 means the absence of lesions. In contrast, degree 4 is the observation of severe lesions with advanced alteration and destruction of the tissue.

RESULTS

Results real-time PCR

A total of 220 samples were processed, of which 195 (88.63%) were diagnosed as positive for IHHNV by real-time PCR. All epidemiological units showed a large number of positive cases: Piura (30/32; 93.75%), North 02 (36/39; 92.31%), North 01 (42/47; 89.36%), South 01 (43/48; 89.58%), South 02 (17/20; 85%), and Center (27/36; 75%) (Table S1).

The cycle threshold (Ct) values of the IHHNV-positive cases were distributed from Ct 14 to Ct 38. Overall, we observed that 39.49% of positive cases presented Ct values <30, followed by 30.77% with Ct values ranged 30-34, while 26.67% showed Ct values ranged 35-37, and only 3.07% of positive cases showed Ct values >37. Regarding the samples selected for histopathological analysis, the highest percentage of positive cases (53.26%) presented Ct values 30-34, followed by 33.70% with Ct values ranging from 35-37. The percentage of positive cases with Ct values <30 was 11.97%, and only 1.08% showed Ct values <37 (Fig. 2).

Histopathology results

Of the 64 *P. vannamei* specimens analyzed by histopathology and H&E technique, all the cases showed typical or related IHHNV lesions (Table S2). In these histopathological lesions, the observation of mild alterations in the nucleus and the presence of eosinophilic Cowdry type A intranuclear inclusions or hypertrophied nuclei, which place the chromatin at the margin of the cells of target tissues such as cuticular epithelium, epicardial epithelium, and connective tissue were mainly visualized. In all cases, the severity of the lesions due to infection by IHHNV was low, and they were categorized as grade 1. There were no samples with lesions compatible with inflammatory reactions or characteristics of moderate or severe infection caused by IHHNV (Table 2).

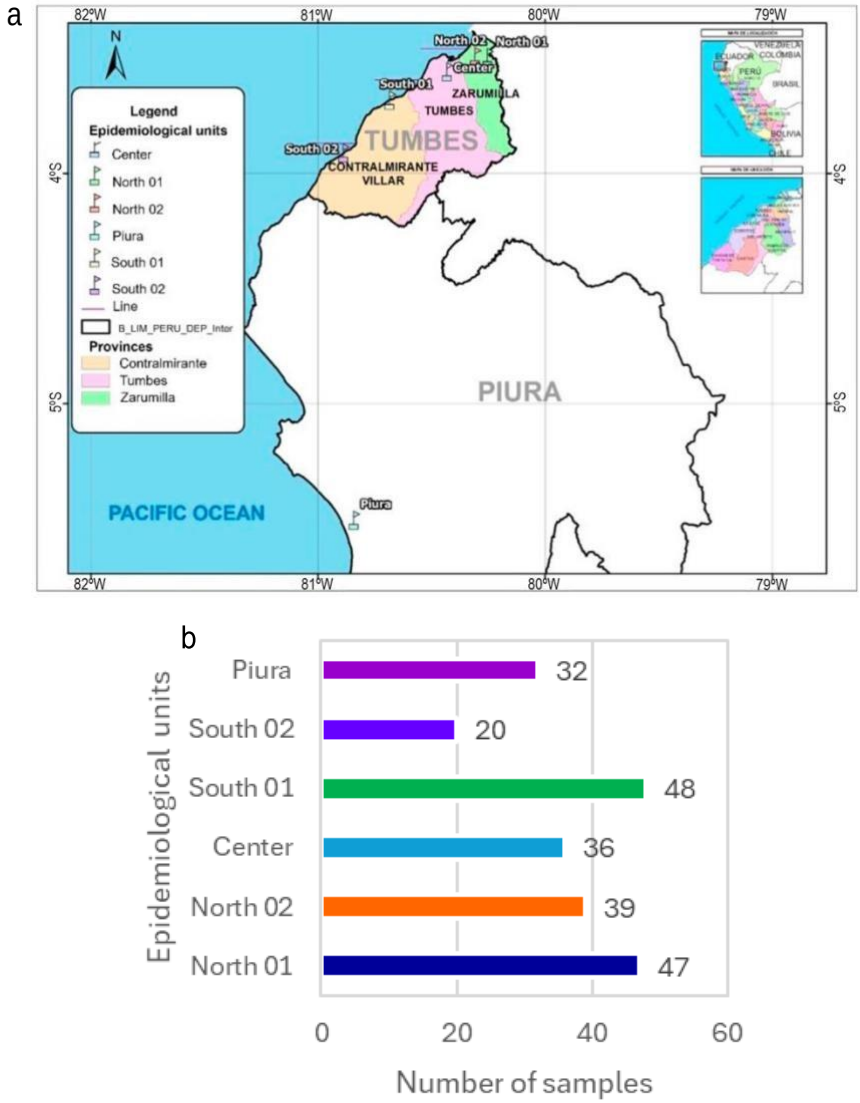

Figure 1. a) Location of the epidemiological units (EUs) sampled, b) number of samples collected. The EUs are distributed along the northern coast of Peru, specifically in the departments of Tumbes (North 01, North 02, Center, South 01, and South 02) and Piura (Piura).

Table 1. List of primers used for the molecular analysis of infectious hypodermal and hematopoietic necrosis virus (IHHNV) in *Penaeus vannamei* samples by PCR. *Endogen control.

Name	Sequences	Amplicon size	Reference
B-actin-F*	CGAGGTATCCTCACCCTGAAAT		
B-actin-R*	GTGATGCCAGATCTTCTCCATGT	81 pb	Jang et al. (2011)
B-actin-Pr*	CGAGCACGGCATCGTCACCAA (FAM-MGB)		
IHHNV1608F	TACTCCGGACACCCAACCA		
IHHNV1688R	GGCTCTGGCAGCAAAGGTAA	81 pb	Tang & Lightner (2001)
IHHNV-Probe	ACCAGACATAGAGCTACAATCCTCGCCTATTTG (FAM-TAMRA)		

Among the most frequent findings are hepato-pancreatic necrosis, gill lamellae hyperplasia, and the presence of hypertrophied and pyknotic nuclei or Cowdry type A inclusion bodies in gill lamellae, epicardial and stomach epithelium. Additionally, inclusion bodies associated with IHHNV were princi-

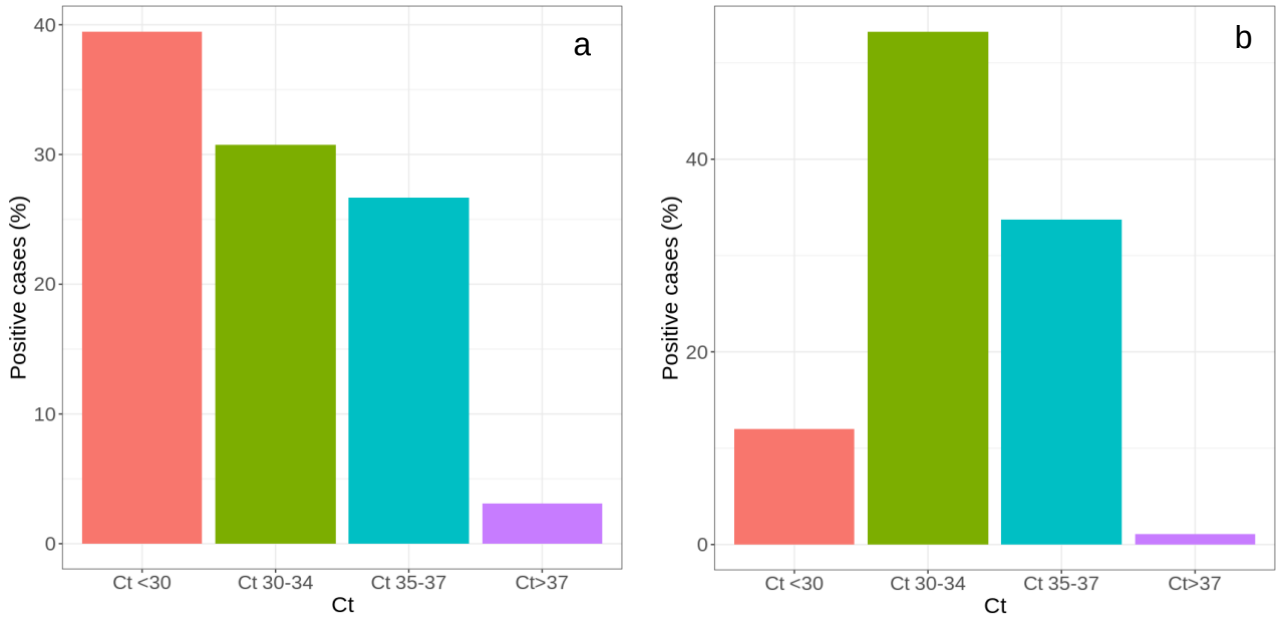

Figure 2. Percentage of infectious hypodermal and hematopoietic necrosis virus (IHHNV) positive cases by cycle threshold (Ct) value ranges. a) Ct values of all positive cases, b) Ct values of positive cases selected for histopathological analysis.

Table 2. Demonstration of the tissue, histopathological description, diagnosis, and frequency of significant histopathology lesions in tissues of *Penaeus vannamei* mainly affected by infectious hypodermal and hematopoietic necrosis virus (IHHNV) and other lesions associated with pathogens like white spot syndrome virus (WSSV), acute hepatopancreatic necrosis disease (AHPND), and necrotizing hepatopancreatitis (NHP) in 2022. [a]Findings associated with IHHNV infection. [b]Findings associated with WSSV infection.

Tissue	Histopathological description	Diagnosis	Frequency
Hepatopancreas	Necrosis and sloughing of tubular epithelium, melanization Hemocytic infiltration, granulomas Structural loss of tubular areas of hepatopancreas	Focal to multifocal, mild to severe, hepatopancreatic necrosis	58/64
Gill	Hyperplasia with lamellae fusion, hemocytic infiltration, melanization Hypertrophied and pyknotic nuclei	Multifocal, mild, gill lamellae hyperplasia	56/64
	Cowdry type A intranuclear eosinophilic inclusions[a]	Focal, mild, karyomegaly[a]	44/64[a]
	Prominent basophilic Cowdry type A inclusion bodies[b]	Focal, moderate to severe, marked karyomegaly[b]	12/64[b]
Cuticular epithelium	Hypertrophied and pyknotic nuclei	Focal, mild, karyomegaly[a]	52/64[a]
	Cowdry type A intranuclear eosinophilic inclusions[a] Prominent basophilic Cowdry type A inclusion bodies[b]	Focal, moderate to severe, marked karyomegaly[b]	20/64[b]
Epicardial epithelium	Hypertrophied and pyknotic nuclei in connective tissue. Cowdry type A intranuclear eosinophilic inclusions	Focal, mild, karyomegaly	22/64
Stomach epithelium	Hypertrophied, basophilic, and pyknotic nuclei Prominent basophilic Cowdry type A inclusion bodies	Diffuse, severe, marked karyomegaly	20/64

pally detected in the cuticular epithelium (Fig. 3), gill, and epicardial epithelium (Fig. 4) without serious injury or association with a proinflammatory immune response; in addition, less frequently, prominent basophilic inclusion bodies were observed in the gill.

Interestingly, viruses were related to the most common pathogenic-potential microbial in all six locations (Center, North 01, North 02, Piura, South 01, and South 02). Thus, 81.25% of the 64 evaluated samples showed at least one type of viral inclusion body with karyomegaly, and 18.75% presented at least two types associated with karyomegaly. IHHNV was the most common virus observed in all regions, with the cuticular epithelium and gill as its principal target organs. Mild to severe lesions in hepatopancreatic tissue, corresponding to 90.63% of the total number of

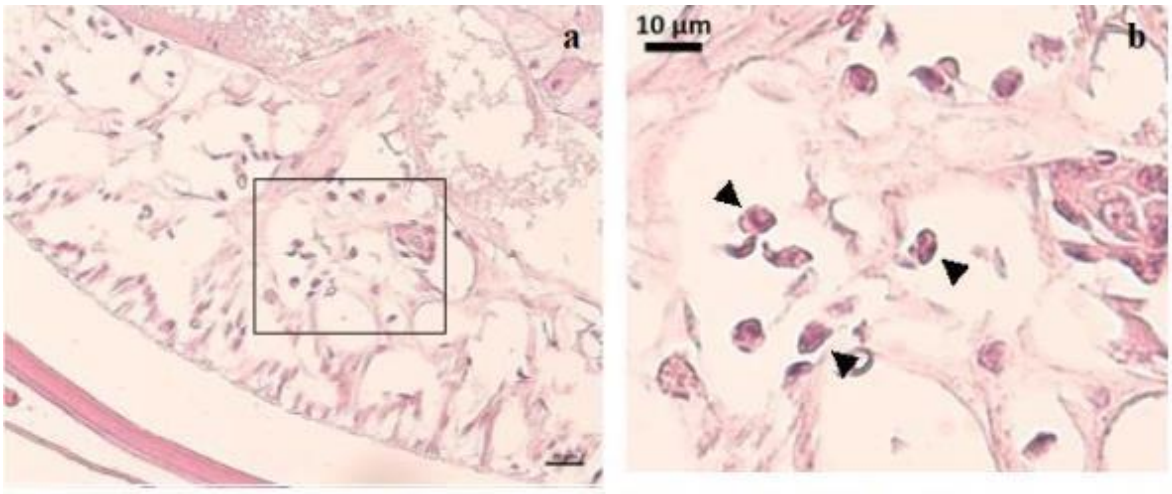

Figure 3. Cultured juvenile shrimp (*Penaeus vannamei*) affected by infectious hypodermal and hematopoietic necrosis virus IHHNV. a) Cuticular epithelium of pleopods (H&E) with focal, mild karyomegaly without signs of inflammatory response visualized at 40x magnification, b) magnification area, arrowheads indicate signs of nuclear degeneration like developing Cowdry type A intranuclear eosinophilic inclusions and hypertrophied and pyknotic nuclei in connective tissue of pleopods.

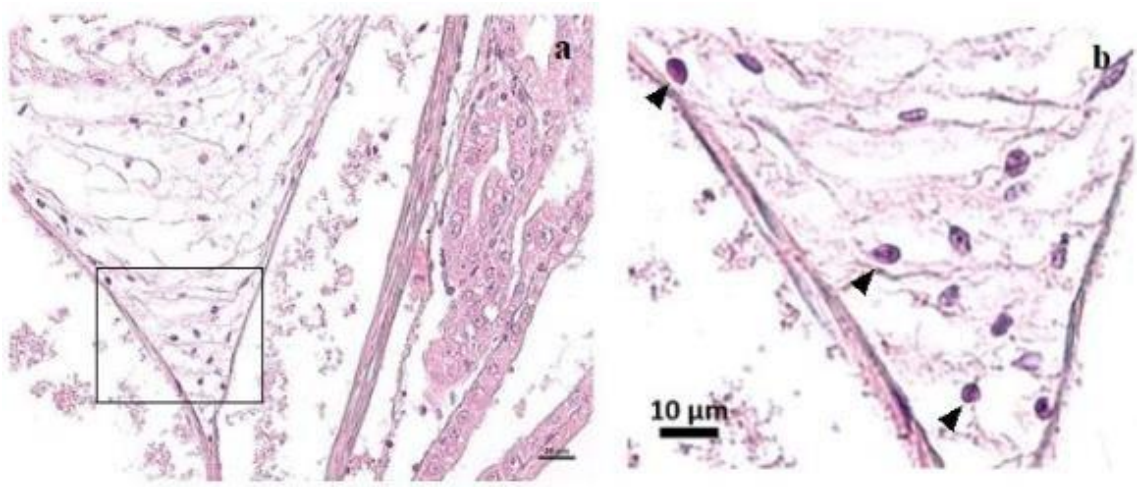

Figure 4. Cultured juvenile shrimp (*Penaeus vannamei*) affected by infectious hypodermal and hematopoietic necrosis virus (IHHNV). a) Epicardial epithelium (H&E) with focal, mild karyomegaly without signs of inflammatory response visualized at 40x magnification, b) magnification area, arrowheads indicate signs of nuclear degeneration like developing Cowdry type A intranuclear eosinophilic inclusions and hypertrophied and pyknotic nuclei in connective tissue of epicardial epithelium.

samples, were mainly associated with acute hepatopancreatic necrosis disease (AHPND) and necrotizing hepatopancreatitis (NHP) bacterial infection.

It is necessary to mention that in WSSV infection, prominent and several basophilic Cowdry type A inclusion bodies accompanied by cytoarchitectural alterations and hypertrophied-pyknotic nuclei were found (Fig. 5). In contrast, bacterial infection compatible with AHPND and NHP disease were characterized by prominent inflammatory response at the sites of histopathological lesions such as hemocytic infiltration, melanization and, granulomas (Fig. 6). Nevertheless. In contrast, all 64 samples analyzed by histopathology were IHHNV positive by PCR technique, and no signs of inflammatory response associated with IHHNV infection, such as hemocytic infiltration or melanization, were found.

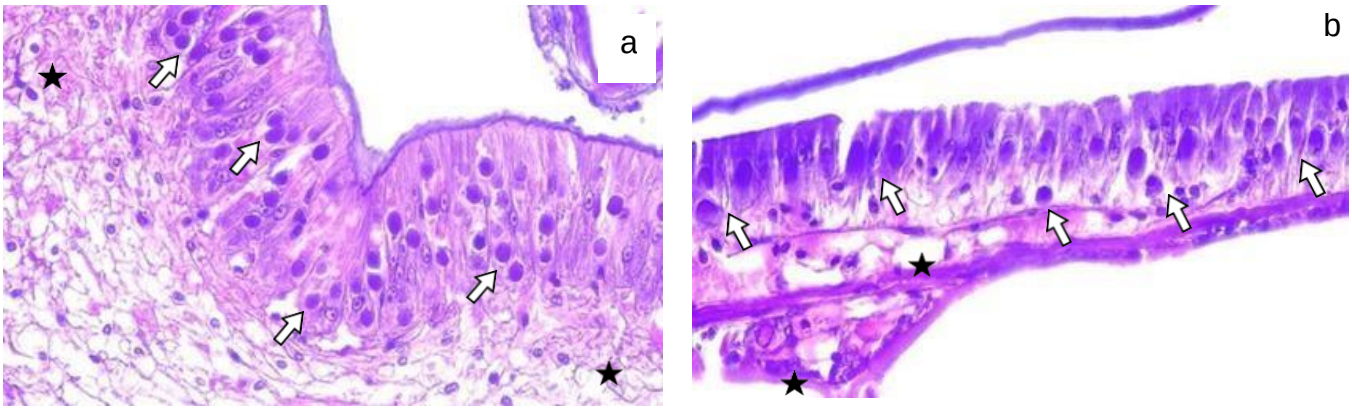

Figure 5. Cultured juvenile shrimp (*Penaeus vannamei*) affected by white spot syndrome virus (WSSV). a) Stomach epithelium (H&E) with diffuse, severe, marked karyomegaly and prominent basophilic Cowdry type A inclusion bodies (white arrows) with signs of inflammatory response in stomach tissue visualized at 40x magnification, b) cuticular epithelium (H&E) with focal, moderate, marked karyomegaly and prominent basophilic Cowdry type A inclusion bodies (white arrows) with signs of inflammatory response in cuticular tissue (black stars) visualized at 40x magnification.

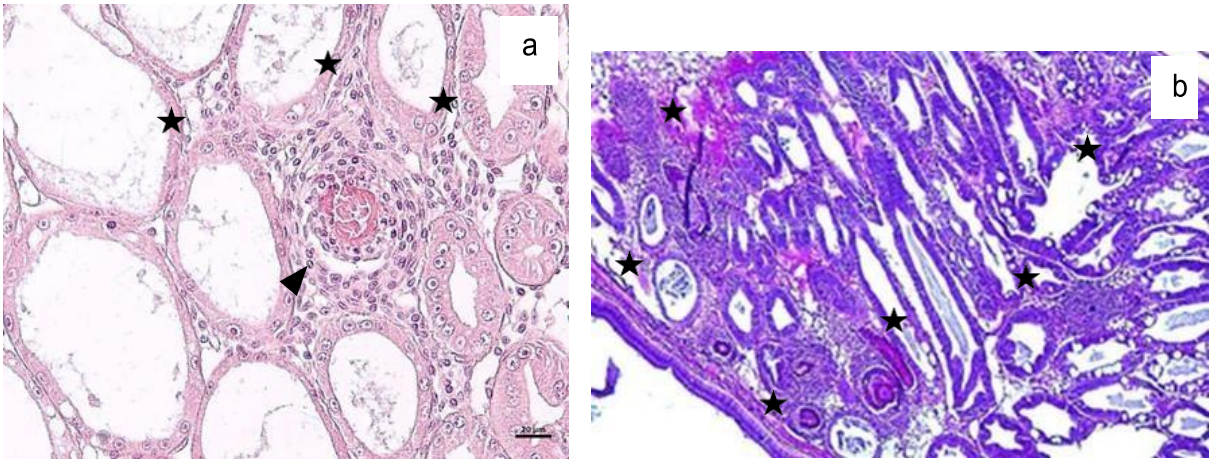

Figure 6. Cultured juvenile shrimp (*Penaeus vannamei*) affected by hepatopancreatic necrosis disease. a) Hepatopancreatic tubules (H&E) with focal, mild necrosis and sloughing of tubular epithelium cells (black stars) with signs of inflammatory response v.g. nodulation process (black arrowhead) visualized at 40x magnification, b) hepatopancreatic tubules (H&E) with diffuse, severe necrosis, sloughing of tubular epithelium cells, structural loss of tubular areas with signs of inflammatory response v.g. several nodulations and melanization foci (black stars) visualized at 40x magnification.

In summary, the results of coinfection cases detected by PCR and histopathology from all six epidemiological units indicate that the center epidemiological unit had the highest range of identified pathogens and associated histopathology observations; the majority was within the hepatopancreas, cuticular epithelium, gill, and stomach epithelium. Interestingly, viral and bacterial presence were widespread in all six epidemiological units (Table 3). Hypertrophied and pyknotic nuclei or Cowdry type A intranuclear eosinophilic inclusions associated with IHHNV were identified in all epidemiological units. However, the lesion severity because of IHHNV infection was mild and therefore classified as grade 1.

DISCUSSION

Infection with IHHNV is a crustacean disease that causes large-scale mortality in *P. stylirostris*, as well as deformities and growth retardation in *P. vannamei* and *P. monodon*. This pathogen is highly prevalent in shrimp farms throughout Latin America (Aranguren et al. 2022). Results obtained in this study show that

Table 3. Epidemiological unit (EU), number of histopathological samples per EU, and PCR and histopathology coinfection cases of *Penaeus vannamei* in 2022. IHHNV: infectious hypodermal and hematopoietic necrosis virus, WSSV: white spot syndrome virus, AHPND: acute hepatopancreatic necrosis disease, and NHP: necrotizing hepatopancreatitis.

EU	Number of histopathology samples per EU	PCR and Histopathology coinfection cases
Center	15/64 (23.44%)	IHHNV (15/15), WSSV (5/15), AHPND (3/15), NHP (3/15)
North 01	13/64 (20.31%)	IHHNV (13/13), WSSV (3/13), AHPND (3/13), NHP (5/13)
North 02	11/64 (17.19%)	IHHNV (11/11), WSSV (3/11), AHPND (2/11), NHP (3/11)
Piura	6/64 (9.38%)	IHHNV (6/6), NHP (1/6)
South 01	16/64 (25.00%)	IHHNV (16/16), WSSV (4/16), AHPND (4/16), NHP (1/16)
South 02	3/64 (4.69%)	IHHNV (3/3), AHPND (2/3), NHP (1/3)

88.63% of the samples analyzed by real-time PCR were positive for IHHNV.

Real-time PCR is the gold standard technique for the molecular diagnosis of IHHNV. The manual of diagnostic tests for aquatic animals of WOAH recommends several real-time PCR methods that differ in specificity, sensitivity, and limit of detection, among other factors evaluated and supported by published studies. These recommendations are taken into account by different agencies that carry out aquaculture epidemiological surveillance. In our case, we used the method of Tang & Lightner (2001), whose detection limit is 10 copies of IHHNV DNA, approximately relating to a Ct value = 38. The Ct value is a crucial factor in interpreting the results of the real-time PCR testing used to determine the presence and infer the magnitude of IHHNV infection in a shrimp population.

Overall, the Ct value provides information about the viral load present in a sample. A lower Ct value indicates more viral genetic material in the sample, suggesting a higher viral load or more active infection. Likewise, a higher Ct value indicates a lower viral load (Tang & Lightner 2001, Cowley et al. 2018). The Ct values of the samples selected for histopathological analysis ranged from 14.27 to 38. Although some Ct values were low and could indicate a higher viral load or active IHHNV infection, it is not related to the results of the histopathological analysis since no inflammatory response was observed (or it was very scarce). No severe IHHNV lesions were observed in the samples analyzed.

The inflammatory response can only be evidenced through drastic alterations in the nucleus that massive Cowdry can generate type A eosinophilic inclusion bodies (Chayaburakulk et al. 2005, WOAH 2023). Thus, we observed scarce or absence of extravasation, hemocyte infiltration, and melanization in tissues with minor signs of nuclear degeneration or few developing and small Cowdry type A corpuscles.

The absence of inflammatory response in tissues and cells of *P. vannamei* has been attributed to the formation of EVEs, which originated after the insertion of parts of the viral genome into the host genome. The immunological role of EVEs has been demonstrated through experimental infections with infectious IHHNV. These elements can give rise to cvcDNA molecules capable of interfering with IHHNV replication, conferring "tolerance" to persistent viral infections in *P. vannamei* (Taengchaiyaphum et al. 2021). This process has been called "viral accommodation" and can occur naturally during the pathogen-host interaction over time (Flegel 2020), so it is likely occurring in Peruvian populations of *P. vannamei*.

Furthermore, this histopathological analysis evaluated various lesions and microbial agents, principally IHHNV, which also correlated with pathogen detection by PCR in *P. vannamei* samples collected from six different epidemiological units in northern Peru. It also describes a large number of different lesions from histopathology findings associated with viral and bacterial infection, mainly related to AHPND, HND, IHHNV and WSSV, and to discern possible similarities or variations because of geographical area, histopathology severity grade and presence of coinfection. This observation also highlighted the importance of characterizing the observation of particular signs of an infection, differentiating the presence of active immune response with histopathological lesions of inflamma-

tion that could trigger a disease (Jiravanichpaisal et al. 2006, Tassanakajon et al. 2013).

Likewise, the description mentioned above of coinfection correlates with the studies of multiple infections in shrimp, presenting a viral, bacterial, or parasitic origin that can affect the ability of shrimp to respond to infection or how they tolerate and can be susceptible to disease. Thus, it has been shown that coinfection between a virus and a bacterium, like the association of AHPND and WSSV, can increase shrimp mortality more than infection by a single pathogen (Han et al. 2019). Also, Teixeira-Lopes et al. (2011) demonstrated a natural coinfection with IHHNV and infectious myonecrosis virus in *P. vannamei*. Moreover, Yu et al. (2011) detected IHHNV and WSSV in six of eight shrimp farm samples, Saravanan et al. (2021) and Macías-Rodríguez et al. (2014) found IHHNV and WSSV coinfection in wild crustaceans from India and México, respectively. Interestingly, viral interference was described in the case of coinfection between IHHNV and WSSV, where both viruses infected the same host cell. Still, one inhibits the replication of the other by competing for protein receptors on the cell membrane (Yan et al. 2016, Yu et al. 2021). Moreover, the interference effect on WSSV is observed when the IHHNV strain is infective because the shrimp develop an active and sustained immune response against IHHNV that also protects them against WSSV infection (Zhang et al. 2016, Escobedo-Bonilla 2021), which demonstrates that the crustacean immune system, once adequately activated, protects shrimp against significant IHHNV infection.

This research presents histopathological results using the H&E technique compatible with the findings of resilience against IHHNV of the study by Aranguren-Caro et al. (2022). However, the results of the present study are not necessarily confirmatory for viral or bacterial identity in shrimp tissues due to a lack of tools such as *in situ* hybridization. For this reason, the laboratory is currently implementing an *in situ* hybridization technique using antisense RNA probes to detect IHHNV viral genetic material in shrimp host tissue.

It is necessary to indicate that the real-time PCR assays were carried out in 2022 with the primers recommended by the WOAH to detect IHHNV. However, in mid-2023, the WHOAH manual was updated based on recent studies indicating that the primers amplify EVE related to IHHNV within the *P. monodon* genome. Considering that *P. monodon* and *P. vannamei* are related species, we performed an *in silico* analysis (results not shown in this article), observing that the primers used can also amplify EVE in the genome of *P. vannamei* (scaffold LVANscaffold_759). According to the results obtained in this study, it is possible to infer that a large part of the amplifications observed in the samples initially diagnosed as "positive" could correspond to EVEs. Therefore, the WHOA recommendations for IHHNV surveillance have been considered in the next epidemiological surveillance plan in 2024.

CONCLUSIONS

The sampled epidemiological units presented a high prevalence of IHHNV-positive cases. However, no histopathological evidence of an inflammatory response was found, and no mortalities attributed to this pathogen were reported on the northern coast of Peru. These observations are related to recent findings on the presence of EVEs in the shrimp genome as an adaptive response to IHHNV infections, leading to the absence of a negative impact on the culture of *P. vannamei*.

Credit author contribution

L. Dominguez-Mendoza: conceptualization, methodology, data curation, formal analysis, writing-original draft; S. Tapia-Chirinos: conceptualization, methodology, formal analysis, writing-original draft; J. Nuñure-Ortega: conceptualization, methodology, data curation, formal analysis, writing-original draft; J. Rodríguez-Callan; methodology and data curation; S. Grabiel-Ataucusi & F. Ramos-Espinoza: methodology, formal analysis, review of original draft and editing; M.M. Gómez-Sánchez & R. Velazco-Peña: conceptualization, methodology, formal analysis, review and editing. All authors have read and accepted the published version of the manuscript.

Conflict of interest

The authors declare no potential conflict of interest in this manuscript.

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