



Practical scoring system for diagnosis presumptive of acute necrosis of the hepatopancreas (AHPND) in shrimp *Penaeus vannamei*

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Received: 3 October 2022 / Accepted: 4 November 2022 / Published online: 13 November 2022
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Abstract

The evaluation of clinical signs is a practical tool commonly used to monitor the health status and detect diseases in shrimp farms. The usefulness of a practical scoring system (PSS) for the presumptive detection of AHPND was evaluated. The presence or absence of food in the stomach and intestine and the changes in the external and internal appearance of the hepatopancreas (Hp) were included. Each criterion and the whole of the PSS were contrasted with the histopathological result (gold test) and evaluated the association of each criterion vs the presence of AHPND and the presence of lesions. We present PSS values ≤ 3 in 77.6% of non-diseased cases, and PSS values ≥ 4 were present in 87.5% of diseased cases. An association ($X^2=83.196$, $df=8$, $p=0.000$) was observed between PSS values vs the presence of AHPND and between PSS values ≥ 5 ($X^2=93.136$, $df=4$, $p=0.000$) vs the development of AHPND lesions. The receiver operating characteristic (ROC) curve showed that the highest pooled sensitivity and specificity (87.5 and 77.6%, respectively) were observed at cut-off point $PSS \geq 4$, but cut-off point $PSS \geq 5$ showed high utility with a positive likelihood ratio (+LR=16) and negative likelihood ratio (-LR=0.28). The absence of content in the intestine and stomach and a discoloured Hp on external and internal observation are useful clinical signs to recognise 87.5% of organisms with hepatopancreas lesions. PSS is proposed as an in situ detection test in shrimp culture for greater certainty when seeking a confirmatory diagnosis for AHPND.

Keywords Clinical signs · Shrimp · Practical scoring system · Presumptive detection AHPND · Sensitivity · Specificity

Handling Editor: Brian Austin

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Introduction

Acute hepatopancreatic necrosis (AHPND) is a bacterial disease that can cause up to 100% mortality in shrimp farms (OIE 2021). This disease is caused by specific *Vibrio* spp. strains, harbouring the plasmid containing the toxic gene *pirAB* (Restrepo et al. 2018; Soto-Rodríguez et al. 2022). In 2010, it was recorded for the first time in the Asian continent (Lightner et al. 2012), severely affecting tiger and white shrimp farming with significant production losses (FAO 2013). In early 2013, the first cases were detected in America North, causing a drop in farmed shrimp production in Mexico (Nunan et al. 2014; Soto-Rodríguez et al. 2015) and subsequent distribution to other countries of Central and South America (Restrepo et al. 2018; Dhar et al. 2019; Peña-Navarro et al. 2020) which caused accumulated economic losses of up to ~4 billion USD for the continent American (Caro et al. 2020).

The shrimp industry has implemented diagnostic tools to mitigate disease impact and spread (Lightner and Redman 1998) to obtain reliable and timely results. Recently, rapid diagnostic methods based on molecular techniques and with high sensitivity in pathogen detection have been developed (Sirikharin et al. 2014; Tsai et al. 2014; Dangtip et al. 2015; Kulabhusan et al. 2017; Yu et al. 2018). Traditional diagnostic methods such as observing clinical signs, microbiology, and histopathology are still used, especially when new diseases appear (Devadas et al. 2019). AHPND is a disease with a high impact on shrimp farming worldwide, for which it is still necessary to know the pathogenesis, conditions associated with the detonation of outbreaks, and evaluation methods for the monitoring and surveillance in situ of farmed shrimp. An opportune diagnosis of the disease in the crop could minimise the economic losses caused by mortalities (Tsai et al. 2014). One of the critical aspects of reducing the introduction or spread of diseases is the constant surveillance of the populations in cultivation through monitoring the organisms' productive parameters, behaviour, and clinical signs (FAO 2013). Observing clinical signs plays a significant role in monitoring the health of organisms (Lightner and Redman 1998).

The clinical and pathological characteristics of AHPND occur mainly in newly seeded organisms during the first 30 days of culture (Tran et al. 2013; Joshi et al. 2014; Nunan et al. 2014). Clinical signs of AHPND include erratic swimming, lethargy, chromatophore expansion, and distinguishable macroscopic changes in shrimp, such as an empty digestive tract and changes in the external appearance of the hepatopancreas (Tran et al. 2013; Soto-Rodríguez et al. 2018; OIE 2021). The necropsy of diseased organisms reveals in the hepatopancreas a change in the colour tone of the organ with different degrees of atrophy and consistency that ranges from very friable to rubbery that is difficult to disintegrate (Soto-Rodríguez et al. 2015; Restrepo et al. 2018). On the contrary, a good swimming activity, cuticle and translucent musculature (without evidence of external lesions such as melanisation, necrosis, or colour changes), stomach and intestine full of food, and hepatopancreas of standard size with dark colouration, that is, well pigmented, are signs that are indicators of a healthy organism (FAO 2009).

The findings recorded from evaluating clinical signs in aquatic organisms represent solid support to establish a diagnosis (Bondad-Reantaso et al. 2001) and guide the necessary analyses to confirm the diagnosis in level II and III laboratories. This work aims to evaluate the diagnostic utility of the main clinical signs of AHPND and to establish a practical scoring system (PSS) for the presumptive detection of applicable disease in shrimp cultures.

Fig. 1 *P. vannamei* showing the criteria for the evaluation of external clinical signs of the stomach and intestine. **a** Photography of shrimp with the full digestive tract (arrow) and **b** empty stomach and intestine (arrowhead)

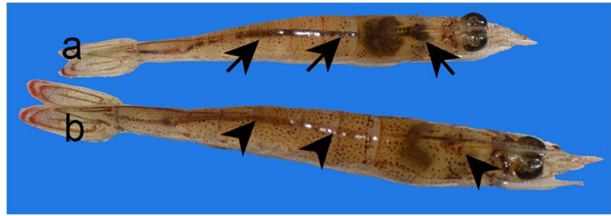
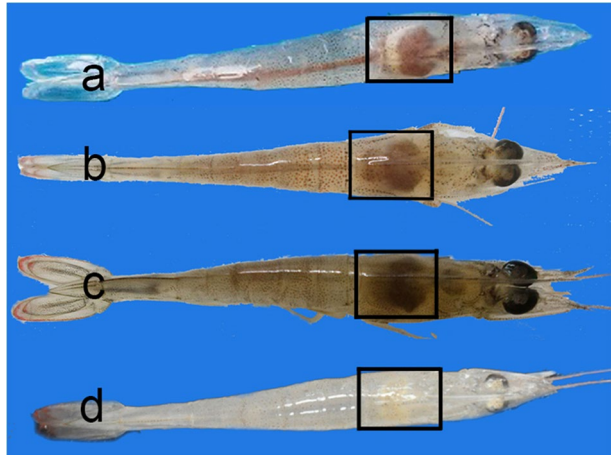


Fig. 2 Shrimp showing the criteria of external appearance changes in the hepatopancreas. **a** Shrimp hepatopancreas with membrane defined-dark (black box); **b** shrimp with hepatopancreas defined-pale (black box); **c** shrimp with hepatopancreas without definition-dark (black box); **d** shrimp with hepatopancreas without definition-pale (black box)



Materials and methods

The study group consisted of 149 records of cases referred for histopathological analysis in the period from 2013 to 2014. Fifty-seven cases correspond to juvenile shrimp *P. vannamei* mean weighing 2.9 ± 1.7 g from six different cultivates of Sinaloa, 48 cases of wild shrimp (3.2 ± 3.0 g) collected in coastal lagoons of Sinaloa, and 44 cases of shrimp with mean weigh 6.8 ± 6.6 g from a bioassay with *Vibrio parahaemolyticus* (*Vp*) causing of AHPND (Soto-Rodríguez et al. 2015). The study cases were taken at random, and inclusion criteria were records that included a photograph of the organism showing external clinical signs, dissection of the hepatopancreas, and histopathological diagnosis. On the other hand, based on the main clinical signs described in diseased shrimp with AHPND (Tran et al. 2013; Soto-Rodríguez et al. 2015) were evaluated the changes in the digestive tract and changes in colouration and external and internal appearance of the hepatopancreas (Hp). The presence (Fig. 1a) or absence (Fig. 1b) of ingested content was observed in the stomach and intestine. The external appearance and colouration of hepatopancreas were classified in four visual characteristics: hepatopancreas with the membrane (Hp c/m)—defined-dark (Fig. 2a), defined-pale (Fig. 2b), without definition-dark (Fig. 2c), and no definition-pale (Fig. 2d).

Finally, the change in colour and internal appearance of the hepatopancreas without membrane (Hp s/m) was classified into four characteristics: dark or pigmented (Fig. 3a), slightly pale (Fig. 3b), pale (Fig. 3c), and whitish-atrophied (Fig. 3d).

A practical scoring system (PSS) was designed to evaluate each previously described characteristic, with a categorical value of 0 or 1 concerning the presence or absence of

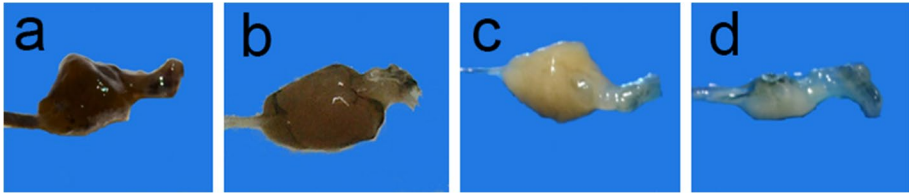


Fig. 3 Hepatopancreas without membrane of shrimp *P. vannamei* showing the criteria for the evaluation of the colouration of the organ. **a** Dark. **b** Slightly pale. **c** Pale. **d** Whitish-atrophied

content in the stomach and intestine. For Hp, categorical values were established from 0 to 3 in the two classifications for Hp c/m and Hp s/m (Table 1).

The histopathological result was considered the gold standard for analysing the PSS criteria. The positive or diseased cases were the organisms with histopathological lesions characteristic of the phases of AHPND (as massive sloughing of epithelial cells, elongation of epithelial cells or necrotic tubules with the proliferation of bacteria associated with tissue necrotic) previously described by Aguilar-Rendon et al. (2020) and Soto-Rodriguez et al. (2022), while cases with histopathological reports without apparent lesions in Hp were considered not diseased.

Both the results of the individual evaluation of each clinical sign and as a whole in the PSS were contrasted with the results of the histopathological analysis (gold test) to evaluate the ability to discriminate diseased cases from healthy ones. The association between the PSS criteria with the result derived from the histopathological diagnosis (healthy/diseased) and with the presence/absence of lesions associated with the AHPND phases was analysed using the Chi-square independence test. The analysis of the area under the curve (AUC) of the ROC curve (receiver operating characteristic) was used to determine the best cut-off point for maximum joint sensitivity (Se) and specificity (Sp) through using the SPSS Statistics 21 programme. Qualitative variables were presented as mean \pm SD and frequency

Table 1 Practice scoring system (PSS) criteria for disease detection

Observation	Clinical signs	Value
Stomach	Full	0
Stomach	Empty	1
Intestine	Full	0
Intestine	Empty	1
Hp c/m	Defined-dark	0
	Defined-pale	1
	No definition-dark	2
	No definition-pale	3
Hp s/m	Dark	0
	Slightly pale	1
	Pale	2
	Whitish-atrophied	3
<i>Total score</i>		8

Hp c/m, hepatopancreas with the membrane; *Hp s/m*, hepatopancreas without the membrane

(percent). A p value <0.05 or less was considered statistically significant. Finally, from the results of curve ROC, the maximum yield was calculated in Se, Sp, positive likelihood ratio (+LR), negative likelihood ratio (-LR), positive predictive values (PPV), and negative predictive values (PNV) using software working in epidemiology (<http://www.winepi.net/>) and Diagnostic Test Calculator (<http://araw.mede.uic.edu/cgi-bin/testcalc.pl?DT=&Dt=&dT=&dt=&2x2=Compute>).

Results

The histopathological analysis showed that 57% (85/149) of the records analysed did not present lesions associated with the disease, considering these cases as negative or not diseased (Fig. 4a), and 43% of the cases (64/149) were diagnosed as positive or diseased due to present hepatopancreatic lesions associated with AHPND phases (Fig. 4b–d and Fig. SI 1 in supplementary information). 77.6% of the non-sick shrimp (66/85) showed external clinical signs with PSS values of 0 to 3 (Fig. 4e), such as a full digestive tract, dark defined Hp c/m and a dark Hp s/m (Fig. 5a and b) or stomach and intestine empty, dark defined Hp c/m and a dark Hp s/m (Fig. 5c). In comparison, 87.5% (56/64) of the diseased cases showed PSS values of 4 to 8, where shrimp with a value of PSS=4 had clinical signs like a digestive tract without food, Hp c/m defined-pale, and a slightly pale colouration of the Hp s/m (Fig. 5d and e); shrimp with a value of PSS=5 showed content in the digestive tract, Hp c/m no definition-pale, and Hp s/m pale (5f); a value of PSS=7 had clinical signs as the empty digestive tract, Hp c/m no definition-pale, and Hp s/m pale (Fig. 5g). The maximal value of PSS=8 includes clinical signs as an empty digestive tract, Hp c/m no definition-pale, and Hp s/m whitish-atrophied (Fig. 5h).

Changes in the appearance of Hp c/m and in colour of Hp s/m were the signs with the greatest association ($X^2 = 59.055$, $df = 3$, $p = 0.000$ and $X^2 = 59.160$, $df = 3$, $p = 0.000$, respectively) vs the presence of AHPND. Table 2 shows the frequency and statistical

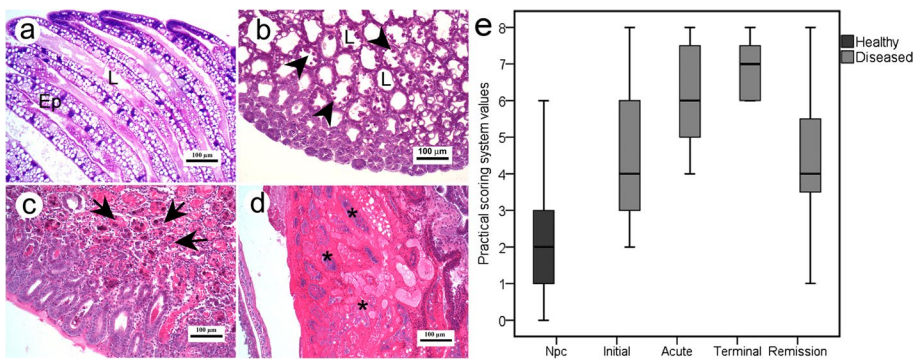


Fig. 4 Photomicrograph of hepatopancreas (Hp) from healthy (a) and diseased (b–d) *P. vannamei* shrimp. a Tubules in longitudinal section with an epithelium (Ep) and tubular structure normal; b–d Hp in transversal cut with lesions associated to ANHPD; Hp in the initial phase (b) the epithelium shows low vacuoles in R, B cells and elongation of the epithelial cells (arrowhead) to the tubular lumen (L); c Hp in the acute phase, the tubules show a massive shoaling and cells necrotic into the tubular lumen (arrow); d Hp in the terminal phase, a severe necrosis tubular and abundant mass of bacteria. Haematoxylin eosin-phloxin stain; e box plot of practical scoring system values (PSS) and change pathological in the hepatopancreas associated with AHPND phases of the diseased group and healthy shrimp. Npc, no pathological changes

Fig. 5 *P. vannamei* shrimp from the healthy group (a–c) and diseased group (d–h). **a** and **b** Shrimp with values PSS = 0. **c** Shrimp with values PSS = 2. **d** and **e** Shrimp with values PSS = 4. **f** Shrimp with values PSS = 5. **g** Shrimp with values PSS = 7. **h** Shrimp with values PPS = 8. The right figure shows the Hp without membrane of each shrimp from left figure

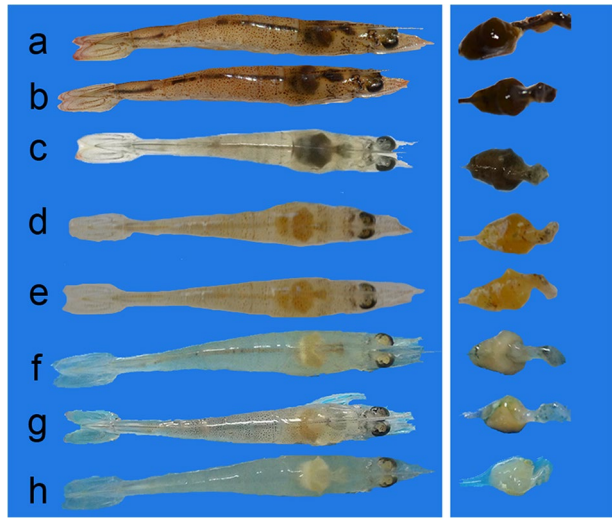


Table 2 Frequency and Chi-square analysis for each evaluation criterion PSS vs histological diagnosis of AHPND

Criterion	Score	Diseased group n = 64		Healthy group n = 85		X ² (gl)	p
		Present n (%)	Absent n (%)	Present n (%)	Absent n (%)		
Empty stomach	1	51 (79.7)	13 (20.3)	21 (24.7)	64 (75.3)	44.197 (1)	0.000
Empty intestine	1	58 (90.6)	6 (9.4)	45 (52.9)	40 (47.1)	24.294 (1)	0.000
Hp c/m appearance change:	-	62 (96.8)	2 (3.1)	49 (57.6)	36 (42.3)	59.055 (3)	0.000
Defined-dark	0	2 (3.1)	62 (96.9)	36 (42.4)	49 (57.6)	29.571 (1)	0.000
Defined-pale	1	9 (14.1)	55 (85.9)	25 (29.4)	60 (70.6)	4.884 (1)	0.027
No definition-dark	2	31 (48.4)	33 (51.6)	24 (28.2)	61 (71.8)	6.399 (1)	0.011
No definition-pale	3	22 (34.4)	42 (65.6)	0 (0.0)	85 (100.0)	34.280 (1)	0.000
Hp s/m colour change	-	56 (87.5)	8 (12.5)	39 (45.9)	46 (54.1)	59.160 (3)	0.000
Dark	0	8 (12.5)	56 (87.5)	46 (54.1)	39 (45.9)	27.367 (1)	0.000
Slightly pale	1	19 (29.7)	45 (70.3)	36 (42.4)	49 (57.6)	2.515 (1)	0.113
Pale	2	17 (26.6)	47 (73.4)	1 (1.2)	84 (98.8)	22.153 (1)	0.000
Whitish-atrophied	3	20 (31.3)	44 (68.8)	2 (2.4)	83 (97.6)	24.225 (1)	0.000

Hp c/m, hepatopancreas with membrane; Hp s/m, hepatopancreas without membrane

analysis of each criterion evaluated versus the presence of the disease. It was observed that a defined-dark Hp c/m and dark Hp s/m were absent in 96.9 and 87.5% of the diseased shrimp (X² = 29.571, df = 1, p = 0.000 and X² = 27.367, df = 1, p = 0.000, respectively), while a whitish-atrophied Hp s/m and Hp c/m without definition-pale were absent in the 97.6 and 100% group of healthy shrimp (X² = 24.225, df = 1, p = 0.000 and X² = 34.280, df = 1, p = 0.000, respectively). Empty stomach and intestine occurred

in 79.7 and 90.6% of diseased shrimp ($X^2=44.197$, $df=1$, $p=0.000$ and $X^2=24.294$, $df=1$, $p=0.000$, respectively) (Table 2).

Data analysis using the ROC curve confirmed that the evaluation criteria have discriminative power to identify diseased shrimp from healthy ones. An empty intestine and stomach showed the lowest AUC value (0.688 and 0.755, respectively) compared to the observed Hp c/m (AUC=0.847). It was observed that within the criteria evaluated, a Hp c/m without definition-pale and a Hp s/m whitish-atrophied showed the highest AUC values (0.672 and 0.644, respectively) (Table SI 1, in supplementary information). A greater discriminative power was observed when analysing all the integrated criteria as PSS, with an AUC value of 0.903 and a 95% confidence interval (95% CI 0.850–0.956) (Fig. 6).

Data analysis of global PSS showed an association significant ($X^2=83.196$, $df=8$, $p=0.000$) between PSS values vs the presence of AHPND (healthy/diseased) and between PSS values ≥ 5 ($X^2=93.136$, $df=4$, $p=0.000$) vs the AHPND phases; even individual comparison showed an association significant ($p<0.05$) in all values PSS vs the presence of AHPND (healthy/diseased), except for PSS values at 4 ($p=0.556$) (Table SI 2 in supplementary information). The score values of PSS above 5 were absent in up to 97% of the healthy shrimp group.

Based on the cut-off value in the ROC curve analysis, the highest sensitivity and specificity conjunct were observed at PSS values ≥ 4 , with a sensitivity of 87.5% and a specificity of 77.6% (Fig. 6, Table 3). PSS values with a cut-off point ≥ 3 showed a better ability to classify diseased cases (sensitivity of 92.2%); however, a greater number of false positives were observed (35/85), yielding a specificity of 58.8%, and a lower +LR (2.24) and -LR (0.13). In contrast, values with a PSS cut-off point ≥ 5 showed the highest AUC (0.844) with the highest values in specificity (95.3%), a likelihood ratio (+LR=16; -LR=0.28), and positive predictive value (92.2%) (Table 3, Fig. 6). The Chi-square analysis shows that an association significant ($p<0.05$) between optimal cut-off PSS values vs the presence of AHPND was observed (Table 3).

Fig. 6 Area under the ROC curve graphic of the practical scoring system (PSS) for detecting AHPND and the non-discrimination line (diagonal line). The arrow indicates the PSS ≥ 4 cut-off point determining the highest conjunct sensitivity and specificity. Arrowhead shows the cut-off points for greater sensitivity (PSS ≥ 3) and specificity (PSS ≥ 5)

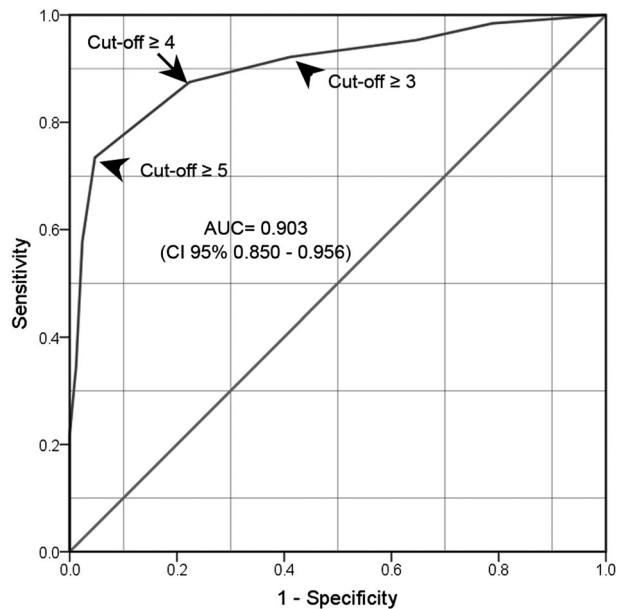


Table 3 Comparison of optimal cut-off values for maximum performance in the practical scoring system (PSS) for diagnostic of AHPND

Cut-off value	AUC (CI 95%)	Se % (CI 95%)	Sp % (CI 95%)	+LR (CI 95%)	-LR (CI 95%)	PPV (CI 95%)	PNV (CI 95%)	χ^2 (gI) p
PSS \geq 3	0.775 (0.677–0.833)	92.2 (85.6–98.8)	58.8 (48.4–69.3)	2.24 (1.72–2.92)	0.13 (0.06–0.31)	62.8 (53.0–72.5)	90.9 (83.3–98.5)	40.796 (1) 0.000
PSS \geq 4	0.826 (0.756–0.896)	87.5 (74.4–95.6)	77.6 (68.8–86.5)	3.91 (2.61–5.88)	0.16 (0.08–0.31)	74.7 (64.8–84.5)	89.2 (82.1–96.3)	61.984 (1) 0.000
PSS \geq 5	0.844 (0.773–0.915)	73.4 (62.6–84.3)	95.3 (90.8–99.8)	16 (5.93–41)	0.28 (0.18–0.42)	92.2 (84.8–99.5)	82.7 (75.2–90.1)	76.613 (1) 0.000

AUC, area under the curve of ROC (receiver operating characteristic curve); Se, sensitivity; Sp, specificity; +LR, positive likelihood ratio; -LR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value

Discussion

One of the critical aspects to consider to reduce the introduction or spread of diseases in shrimp farms is the constant surveillance of the farmed populations (FAO 2013). The evaluation of clinical signs is a commonly used diagnostic tool that allows monitoring the state of health by identifying clinical manifestations that may eventually be suggestive of disease. For the present study, an overall positive rate of 43% (64/149) AHPND was observed, which may be comparable with the positivity rates found in shrimp cultures (Nguyen et al. 2021).

The evaluation of clinical signs by PSS for the presumptive diagnosis of AHPND in white shrimp showed significant differences between PSS vs AHPND values ($X^2=83.196$, $df=8$, $p=0.000$); therefore, there is an association between clinical signs used in PSS and the presence of AHPND. PSS values ≤ 3 were associated with healthy shrimp ($X^2=40.796$, $df=1$, $p=0.000$), that is, a full digestive tract, Hp c/m defined-dark as external appearance, and a dark Hp s/m were indicative of healthy shrimp, while PSS values ≥ 4 were present in diseased shrimp ($X^2=61.984$, $df=1$, $p=0.000$). It is known that the manifestation of clinical signs is related to the presence and evolution of hepatopancreatic lesions (Soto-Rodríguez et al. 2018, Aguilar-Rendon et al. 2020). AHPND disease causes severe hepatopancreas damage with the rapid development of lesions and progression of AHPND phases (Tran et al. 2013; Soto-Rodríguez et al. 2015). Organisms classified by the gold test as diseased (true positives) with characteristic lesions in the acute and terminal phase had a PSS value ≥ 5 , represented by an empty digestive tract, Hp c/m without pale definition, and a marked discolouration of Hp s/m (pale or whitish-atrophied colouration) coinciding with the external appearance of AHPND diseased organisms reported by Lightner et al. (2012), Tran et al. (2013), and Soto-Rodríguez et al. (2015).

Soto-Rodríguez et al. (2018) report clinical signs such as lethargy and anorexia from the first hours after inoculation, subsequently, the absence of content in the digestive tract and the discolouration of the hepatopancreas, which becomes more acute as the exposure time elapses. Aguilar-Rendon et al. (2020), in their study with two strains of *Vp* AHPND+, observed that the manifestation of clinical signs, degree of severity, and progression of the AHPND phases was dependent on time and the virulence of the strain. In the present study, although PSS values ≥ 5 showed an association ($X^2=93.136$, $df=4$, $p=0.000$) with the AHPND phases, the remission phase influences the increased variability in the PSS value because it is usually identified in organisms in recovery. The stomach and intestine presence of content was also related to this variability since this characteristic was observed in shrimp in the remission phase (Soto-Rodríguez et al. 2022), which was classified as false negatives. There is evidence that shrimp experimentally exposed to the *Vp* AHPND+ strain managed to survive the disease and enter a recovery stage (remission), where some shrimp managed to reduce the necrotic lesions (from G3 to G0) caused by intoxication (Aguilar-Rendon et al. 2020; Caro et al. 2020). These shrimp, which tend to reduce lesions, also show improvement in food intake as the exposure time elapses, favouring the accumulation of food content in the digestive tract (Aguilar-Rendon et al. 2020).

On the other hand, the change in the external appearance of the hepatopancreas and the absence of food in the digestive tract observed in non-diseased organisms with PSS values > 3 , in addition to being associated with this and other diseases (Lightner 1996; Vincent et al. 2004; Soto-Rodríguez et al. 2010), could also be associated with handling conditions, for example, prolonged periods without feeding or late analysis time. There are studies in shrimp where a time of 2 to 4 h has been determined for a complete evacuation

of the intestine after feeding (Dall et al. 1990; Nunes and Parsons 2000). In this study, the external characteristics observed in the group of organisms classified as false positives could be due to the starvation period or the moulting process since no other pathology was observed in the organisms. Therefore, it is necessary to consider transport handling and analysis time since they could influence the increase in false positives.

The results of the ROC curve showed that the analysis criteria used in this study have null to moderate discriminative power when each criterion is used separately (AUC = 0.292 to 0.775), unlike when they are used together as PSS because the discriminatory power to identify diseased vs healthy shrimp is increased (AUC = 0.903) with a diagnostic accuracy of 81.8%. It is known that AUC values = 1.00 indicate perfect discrimination of sick vs healthy cases (Cerdeira and Cifuentes 2012); therefore, the use of PSS shows a good discriminatory capacity. There are no comparable studies where clinical signs are evaluated as a continuous test in diagnosing diseases in shrimp; however, some studies based on molecular methods have reported diagnostic accuracy greater than 90% (Tsai et al. 2014; Kulabhusan et al. 2017; Yu et al. 2018). Yu et al. (2018) evaluated the relative abundance of 10 gut disease-discriminatory phyla in shrimp using the qPCR technique with an accuracy between 92.2 and 95.1% in diagnosing bacterial species and phylum levels. Tsai et al. (2014), for their part, obtained a diagnostic accuracy of 95% with a sensitivity of 93.5% and specificity of 97% when using a rapid test for the detection of WSSV. Recently Li et al. (2022) reported slightly lower sensitivity (92.5%) but 100% specificity when using a new molecular assay to detect pirA^{VP} and pirB^{VP} . The present study had a slightly lower PSS test performance (> 75%), with PSS values ≥ 4 with the highest conjunct sensitivity and specificity. Nevertheless, PSS cut-off values ≥ 5 showed high specificity (95.3%), similar to that reported in rapid tests for the detection of WSSV (Tsai et al. 2014). The PPV was also higher (92.2%); that is, a PSS result ≥ 5 has a 92.2% probability of actually being a diseased organism, and during, a PSS result ≤ 3 has a 90.9% probability (PNV) of being a healthy organism. These results are slightly higher than those recorded using a rapid test (PNV = 87%) based on fluorescent signals detecting pirA^{VP} and pirB^{VP} (Li et al. 2022). The use of PSS shows promising results for monitoring the health status of shrimp in culture.

In the present study, the results show that the evolution of the disease and the degree of lesions could influence the detection of false negatives (12.5%), causing a lower capacity of the test to classify diseased organisms correctly. In this context, it is possible to improve the sensitivity of PSS by considering other additional criteria based on the clinical history of the culture, such as the time of evolution of the outbreak, the presence of mortality, and the size of the organisms.

Conclusions

The results showed that the analysis criteria used in this study as PSS have discriminative power to identify organisms with AHPND vs healthy shrimp with high diagnostic accuracy (81.8%). Contrast study with other bacterial diseases that affect the hepatopancreas, such as hepatopancreatic necrosis (NHP-B), septic hepatopancreatic syndrome (SHPS), and common vibriosis, is still pending. The results show that PSS values ≤ 3 is significantly associated with shrimp without lesions in hepatopancreas, while PSS values ≥ 4 were significantly associated with diseased shrimp. Therefore, PSS is proposed as an in situ detection test whose benefits include low cost; it does not require expensive equipment or materials and feasibility in its implementation in shrimp farms to minimise economic losses due to

disease and provide greater certainty when seeking to obtain a confirmatory diagnosis for AHPND.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10499-022-01020-4>.

Acknowledgements This work was supported by the National Fishery and Aquaculture Institute (INAPESCA) and contributions from shrimp farmers.

Author contribution Conceptualisation, methodology, formal analysis, and writing—original draft preparation: Rodolfo Lozano-Olvera; Writing—supervision, review, and editing: Selene Maria Abad-Rosales. All authors read and approved the final manuscript.

Data availability Data generated during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval All procedures were performed according to Mexican guidelines and policies in the NOM-062-ZOO-1999 and British guidelines for fish welfare reported by Ashley (2007).

Competing interests The authors declare no competing interests.

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