Survey On Pseudomoniasis in Cultured *Litopenaeus vannamei* Shrimp Collected from Suez Governorate, Egypt

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Abstract

This current study was applied for investigating the presence of *Pseudomonas* species bacteria from cultured marine shrimp (*Litopenaeus vannamei*) collected from the Suez Governorate randomly and seasonally during the period from March 2020 to November 2020. Our results revealed that the suspected isolated bacteria, was identified as *Pseudomonas fluorescence* depending on both morphological and biochemical characters using traditional as well as API20E commercial tests. The clinical signs appeared on the infected shrimp were melanization on the body surface and darkening of appendages tips, inflamed tail, rostrum deformity, darkened gills and paleness of hepatopancreas. The total prevalence of *Pseudomonas* infection was 17.33%, while the highest seasonal prevalence was recorded in the summer season (20%) followed by autumn season (18%) and the lowest percent was the spring season (14%). The histopathological examination revealed the presence of haemocytic filtration, sloughing hepatopancreas cells, necrotic inflammation, haemocytic plug, broken musculature and epithelial edema. We concluded that higher temperature is the predisposing factors for *Pseudomonas* infection.

Keywords: API20E, *Litopenaeus vannamei*, *Pseudomonas fluorescens*.

Introduction

Shrimps are highly-priced seafood that may be found worldwide in tropical and warm-temperature coastal waters. In many parts of the world, shrimp fisheries provide economic assistance ***Ajani et al. (2013)***. The most popular economically cultured shrimp species worldwide is *Litopenaeus vannamei*. Crustacean aquaculture comprises 57% of the world

brackish water culture, in which marine decapod shrimps, primarily comprising *Penaeus monodon* and *Litopenaeus vannamei*, accounted for more than 99% of total production in 2010 ***FAO (2012)***. Shrimp are exposed to infections that they might not normally encounter when they are moved from their natural offshore or estuarine environment to terrestrial earthen ponds ***Walker et al. (2009).*** In the cultured Pacific white shrimp, *Penaeus vannamei*, bacterial infections had become a significant economic burden that affected survival and growth ***(Avarre et al., 2003; Toranzo et al., 2005; Stentiford et al., 2012; Cao et al., 2014)***. *Vibrio sp.* are the dominant bacteria that infect shrimp eggs, larvae and post-larvae followed by *Pseudomonas, Alcaligenes, Aeromonas* and filamentous bacteria, such as *Flavobacteria, Leucothrix, Thiotrix, Flexibacter* and *Cytophaga* ***(Sunaryanto and Mariam, 1986; Hameed, 1993; and Mourino et al., 2008)***. *Pseudomonas* species have been a dominant microorganism in aquaculture systems ***Sombatjinda et al. (2011)***. *Pseudomonas spp.* produce bioactive compounds with the ability to control *Vibrio's* such as *V. harveyi* and *V. parahaemolyticus*, and that do not affect the shrimp ***Rattanachuay et al. (2007)***. *P. fluorescens* is gram-negative bacteria of the family *Pseudomonadaceae,* which is a pathogen for shrimp ***Swain et al. (2007)***. Genus *Pseudomonas* is widely distributed in soil and aquatic habitats including shrimp ponds ***Sakami et al. (2008)***. Pathogenicity of *Pseudomonas* bacteria is largely dependent on flagella and fimbriae, pyocyanin (PYA), pyoverdine, alkaline proteases, protease IV, elastases, and rhamnolipids production ***Al-Wrafy et al. (2017*)** because they have high adaptation capacity to unfavorable environmental conditions ***Abdullahi et al. (2013)***. *Pseudomonas spp.* occurring in high numbers in fresh and seawater, also have been identified as the cause of diseases in many animals and humans ***Liu et al. (2014)***. Consequently, this study aimed to investigate presence of *Pseudomonas spp.* among infected cultured shrimp, record the clinical and postmortem lesion of the infected samples, record the total and the seasonal prevalence of the disease among examined shrimp and record the histopathological picture of the disease on the affected samples

Materials and Methods

1. Samples collection:

A total number of alive and freshly dead 150 cultured white leg shrimp (*L. vannamei*) of different body weights and lengths with an average body weight of 0.7-7.39 gm. and length of 4.6 - 10.7 cm were collected randomly and seasonally from private shrimp farms at Suez governorate from March 2020 to

November 2020**.** A live samples were transferred in tanks supplied with an air-blower to the Fish diseases laboratory, National Institute of Oceanography and Fisheries, Suez and Aqaba Gulfs Branch, Suez governorate for full clinical, postmortem, bacteriological and histopathological examinations.

2. Clinical and postmortem examination:

Clinical examination and postmortem examination of infected shrimps were performed according to the method described by ***Austin and Austin, (2007)*** for detecting abnormalities.

3. Bacterial isolation and identification:

The isolation of *Pseudomonas* species was achieved from shrimp samples (alive and freshly dead) under the complete aseptic condition on trypticase soya broth (2.5% NaCl, PH 8.6) and incubated at 25ºC for 24-48 hrs, then streaked on *Pseudomonas* isolation agar then incubated at 25ºC for 24-48 hrs. Suspected colonies sub-cultured on TSA (2.5% NaCl) were subjected to microscopic and biochemical analysis, according to ***Buller, (2004).*** Isolates of *Pseudomonas sp.* were kept in stock cultures maintained in tryptic soya broth with 30% glycerol at −80◦C according to ***Nair et al., (2015)***

4. Biochemical identification: Biochemical identification was performed according *to* ***Holt et al., (1994)*** for pure colonies only given gram-negative, oxidase-positive and catalase-positive. The rest of these tests were performed by API 20E strips (BioMerieux, France).

5. Histopathological examination: Specimens for the histopathological test were freshly taken from affected organs organs (hepatopancrease, musculature, gills and guts) of naturally infected shrimps. Specimens were trimmed and fixed in 10% phosphate-buffered formalin and washed with running tap water for 24hrs. The samples were then dehydrated in gradients of ascending alcohol concentrations and cleared with xylol and embedded in paraffin wax to support the tissue for thin sectioning (5-micron thickness). Sections were stained with hematoxylin and eosin (H&E) stain and then examined microscopically by Leica Icc50 HD microscope according to ***Roberts, (2001)*.**

Results

1. **Clinical and Postmortem examination:**

Clinical examination of infected *L. vannamei* shrimps revealed black or brown cuticular erosions with inflamed tails and paleness of the hepatopancreas (Figure 1). Some cases revealed melanization and darkening of appendages tips (Telson) and heavily darkened gills (Figure 2). Others showed deformity of the rostrum with opaque of the abdominal musculature (Figure 3). These examined samples were used for

the detection of causative agents for such lesions.

2. Morphological examination:

Bacterial colonies that appeared on *Pseudomonas* isolation agar were yellowish colonies at 27ºC for18-24 hrs while on Tryptic soya agar supplemented with NaCl (2.5%) were yellowish opaque, round, convex, smooth-edged, and semi-translucent colonies. These colonies are suspected to be *P. fluorescens* according to Table (1).

2. Biochemical identification:

The results of the conventional and commercial systems for biochemical tests revealed that isolates were negative for OPNG, LDC, ODC, H_2S, URE, TDA, IND, VP, GEL, MAN, INO, SOR, RHA, SAC, AMY, and ARA. While they were positive for ADH, CIT, MEL, oxidase, and catalase. While they were (+/-) for GLU. The code numbers on API 20E strips were 2204044 & 2200044 with an accuracy percent about 67% & 90.5% respectively (Figure 4 A & B). According to these results at Table (2), the bacterial isolates were identified as *P. fluorescens*

3. The total and the seasonal prevalence of *P. fluorescens* among infected shrimp:

The total prevalence of *P. fluorescens* was 17.33% while the seasonal prevalence was the highest in summer (20%) followed by autumn (18%) and spring (14 %) (Figure 5).

4. Histopathological examination:

The results of histopathological examination of infected *L. vannamei* shrimp revealed that the hepatopancreas was with an abnormal lumen and sloughing hepatopancreas cells (Figure 6). While the gills of infected shrimp showed separation of cuticle with epithelium edema (Figure 7). Also, the musculature showed broken muscle fibres and haemocytic infiltration (Figure 8 A & B). Finally, the subcuticular gut epithelium appeared with necrotic inflammation, haemocytic plug and haemocytic infiltration (Figure 9).

Table (1): *Showing morphological and cultural characteristics of suspected P. fluorescens isolated from infected L. vannamei shrimp:*

Tests	*P. fluorescens*
Gram stain reaction	**-ve**
Shape	**Short rod**
Arrangement	**Scattered**
Motility	**Actively motile**
Growth on *Pseudomonas* isolation agar	**Yellowish colonies**
Growth at:	
-25-27°C	**+**
-37°C	**+**
-40°C	**+**
Growth on tryptone soya broth with:	
-0% NaCl	**-**
-3% NaCl	**+**
-8% NaCl	**+**

Table (2): *Showing biochemical characteristics of suspected P. fluorescens recovered from suspected L. vannamei shrimp:*

Test of API20E	*P. fluorescens*
B-Galactosidase production (ONPG)	-
Arginine dihydrolase production (ADH)	+
Lysine decarboxylase production (LDC)	-
Ornithine decarboxylase production (ODC)	-
Citrate utilization(CIT)	+
H2S production (H2S)	-
Urease production (URE)	-
Tryptophane deaminase production (TDA)	-
Indol production (IND)	-
Acetoin production (VP)	-
Gelatinase production (GEL)	-
Acid from:	
Glucose (GLU)	+/-
Mannitol (MAN)	-
Inositol (INO)	-
Sorbitol (SOR)	-
Rhaminase (RHA)	-
Sucrose (SAC)	-
Melibiose (MEL)	+
Amygdalin (AMY)	-
Arabinase (ARA)	-
Cytochrome oxidase (OX)	+
Catalase	+

Figure (1): Infected *L. vannamei* shrimps with *Pseudomonas* species showing: Black or brown cuticular erosions (a) with inflamed tail fan (b) and paleness of hepatopancreas (c).

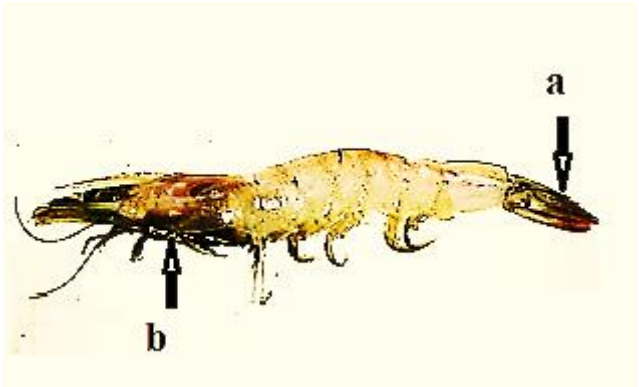

Figure (2): Infected *L. vannamei* shrimps with *Pseudomonas* species showing: Melanization and darkening of appendages tips (Telson) (a) and gills (b).

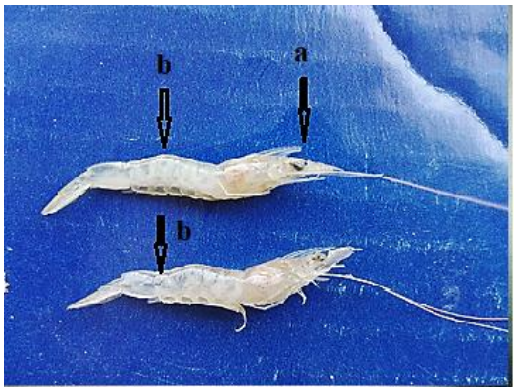

Figure (3): Infected *L. vannamei* shrimps with *Pseudomonas* species reavled: Deformity of rostrum (a) with opaque of the abdominal musculature (b).

Figure (4): Showing suspected *P. fluorescens* on API 20E strips.

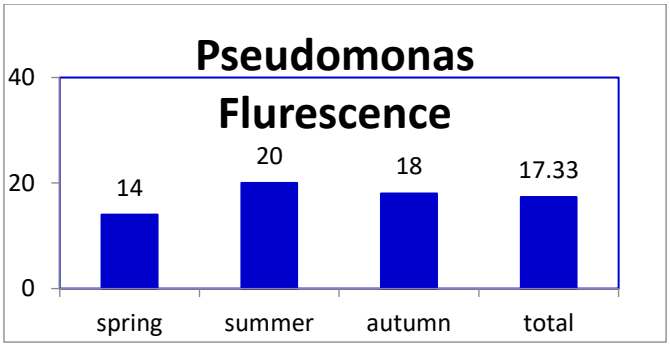

Figure (5): Showing the total and seasonal prevalence of *P. fluorescens* in infected *L. vannamei* shrimp.

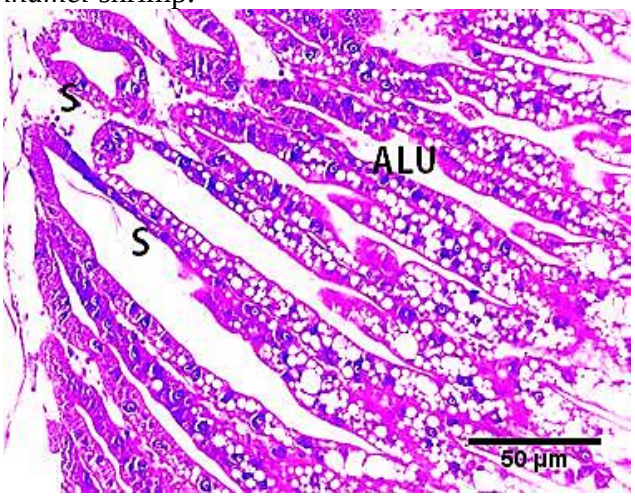

Figure (6): Showing Infected *L. vannamei* shrimp hepatopancreas with an abnormal lumen (ALU), sloughing hepatopancreas cells (S).

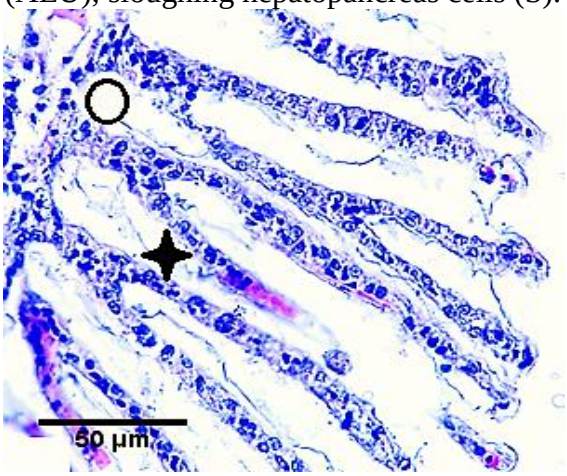

Figure (7): Showing Infected *L. vannamei* shrimp Gills with cuticle separation (Circle) and epithelium edema (Star).

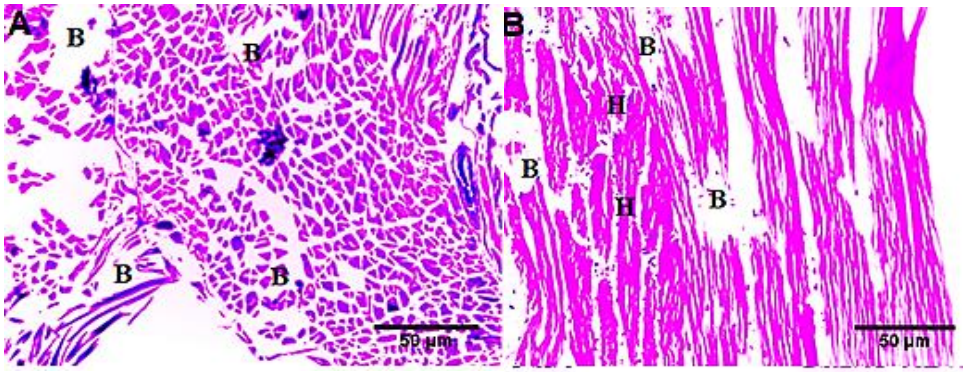

Figure (8): Showing Infected *L. vannamei* shrimp **(A)** Musculature (transverse section) showed broken muscle fibres (B). **(B)** Musculature (longitudinal section) showed broken musculature fibres (B) and haemocytic infiltration (H).

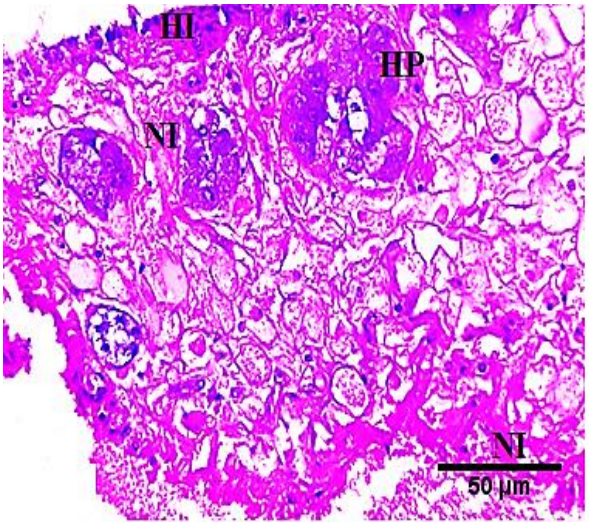

Figure (9): Showing Infected *L. vannamei* shrimp Subcuticular gut epithelium with necrotic inflammation (NI), haemocytic plug (HP) and haemocytic infiltration (HI).

Discussion

In the present study, the clinical signs and postmortem examination of infected *L. vannamei* shrimp revealed black or brown cuticular lesions with inflamed tail and paleness of hepatopancreas, Some cases revealed melanization and darkening of appendages tips (Telson) and heavily darkened fouled gills, while others showed deformity of rostrum with opaque of the abdominal musculature, And that nearly similar to ***Aly and El-Attar, (2001)*** who recorded necrotic foci on the hepatopanceas and intestine along with darkness of the appendages and exoskeleton due to *P. fluorescens* in infected freshwater shrimp in Egypt . These

results may be attributed to bacterial toxins and extracellular products ***Hadi et al. (2012)***. Concerning the total and seasonal prevalence of *P. fluorescens*, our results revealed that the total prevalence was (17.33%) which was (14 %) in the spring season followed by the autumn season (18%) and the summer season (20%). These results were lower than ***Dehkordi et al. (2021)*** who reported the prevalence of *Pseudomonas* species from shrimp samples collected from the wild in Isfahan and Chabahar (Iran), in the summer and the autumn seasons were 48.6% and 22.9%, respectively (with a significant difference between them) while in Chabahar were 25.7% and 20%, respectively (without a significant difference between them). The differences in the prevalence rates of *Pseudomonas* species may be attributed to temperature changes which are one of the factors that cause outbreaks of waterborne diseases ***Tang et al. (2014)*.**

Concerning the results of the bacteriological examination of infected *L. vannamei* shrimp, that appeared on *Pseudomonas* isolation agar were yellowish colonies, also catalase and oxidase-positive, they have revealed the presence of *P. fluorescens* by it confirmed by bacteriological examination which matched with ***Foysal et al. (2011)*** who found that Phenotypic properties of the *P. fluorescens* isolates were gram-negative, rod-shaped and catalase & oxidase positive and ***Raghuraman et al. (2013)*** who found that biochemical tests revealed that isolates were negative for indole production, citrate utilization, glucose fermentation, sucrose fermentation, lactose fermentation, coagulase, urease, esculin hydrolysis and nitrate reduction. While they were positive for motility, methyl red, Voges-Proskauer, H_2S production oxidase and catalase.

Our results concerning the histopathological examination of infected *L. vannamei* shrimp; hepatopancreas showed abnormal lumen, sloughing hepatopancreas cells. Gills had cuticle separation and epithelium edema. Musculature showed broken muscle fibers and haemocytic infiltration. The subcuticular gut epithelium showed necrotic Inflammation, haemocytic plug and Haemocytic infiltration. These results agreed with the study conducted by ***Aly and El-Attar, (2001)*** who recorded that the gills of infected cultured shrimp with *P. fluorescens* showed cellular vacuolation in the anemic primary filaments and epithelial desquamation in the secondary filaments. The gill arch contained numerous hemocytes. In the musculature, edema, congestion, hemorrhage, and hyaline degeneration were evident. Other musculature bundles were necrotic and infiltrated by mononuclear cells. In the hepatopancreas, the hepatic tubules exhibited either

necrotic or ruptured cells and were infiltrated by hemocytes, mononuclear cells and melanomacrophages. The gut revealed marked necrosis in their epithelium with some hemocytes in the submucosa. These results are attributed to the septicemic and /or toxemic picture of these bacteria.

Conclusion

Based on what was clarified by this study, *Pseudomonas fluorescens* . It poses a serious threat to shrimp farming especially in summer season. It could be as a result stresses which increase the infection. So, the farmers should enhance the immune system of the population by using different immunostimulants as an attempt to reduce the mortality rate in high temperatures due to bacterial infections.

Acknowledgment

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الملخص العربي

دراسة حقلية عن مرض الزودوموناسيز في الجمبري الفانمي (الجمبري ذو الأرجل البيضاء) المستزرع في محافظة السويس، مصر

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أجريت هذه الدراسة لفحص أنواع بكتيريا الزودوموناس في مزارع الجمبري الفانمي البحري في محافظة السويس بمصر. تم تجميع عينات الدراسة بشكل موسمي وبطريقة عشوائية في الفترة ما بين مارس 2020 إلى نوفمبر 2020. تم تعريف البكتيريا المعزولة بالطرق المتعارف عليها ظاهريا ومعمليا وذلك بإستخدام النظام الكيميائي الحيوي لتحديد وتمييز الأنتيروباكتيريا أنها زودوموناس فلوريسينس بكتيريا. وقد أظهر الفحص الإكلينيكي للجمبري المصاب ظهور تصبغات سوداء ودكانة وإحمرار في الذيل وشحوب الكبد البنكرياسي. وقد أظهرت النتائج أن نسبة الإصابة الكلية للجمبري المالح 17.33% و ظهرت النسبة الأعلى إنتشاراً في موسم الصيف بنسبة 20% تلاه موسم الخريف بنسبة 18% وكان موسم الربيع أقل نسبة بقيمة 14%. أظهر الفحص النسيجي لعينات الجمبري المصابة وجود ترشيح دموي وتهتك في العضلات وإستسقاء في الأغشية الداخلية.