Preliminary Study of *Vibrio parahaemolyticus* Infection in Shrimp

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Abstract

The present study investigated *Vibrio parahaemolyticus* infection in shrimp. A total of 150 freshly moribund whiteleg shrimp (*Penaeus vannamei*) were collected randomly from various fishermen and various locations in Ismailia Governorate, Egypt, and subjected to clinical, postmortem and bacteriological examinations. Following bacteriological analysis, about 22 *V. parahaemolyticus* isolates were recovered from the examined samples. The infected shrimp exhibited observable clinical signs such as eroded cuticle, brownish to black patches scattered all over the body surface, sloughing of the abdominal appendages, dark and reddish discoloration on pleopods, periopods, carapace and tail area. The most common postmortem findings were whitish musculature, and the hepatopancreas in most cases was either congested or atrophied. On TCBS agar media, *V. parahaemolyticus* exhibited green or bluish green centered colonies. Microscopically, the recovered isolates were Gram-negative, straight or slightly curved short rods and arranged singly or in chains. All the retrieved isolates were negative for the ONPG test and citrate utilization test, while, positive for oxidase, catalase, lysine decarboxylase, ornithine decarboxylase with alkaline slant, acidic butt for TSI test. Our research emphasizes the critical shrimp health threats posed by *V. parahaemolyticus*.

Keywords: *Vibrio parahaemolyticus*, *Penaeus vannamei*, Clinical and postmortem examinations.

Introduction:

Shrimp are a significant and valued seafood choice for people globally ***(Otwell et al., 2001)***. Over the past three decades, shrimp farming has expanded in tropical and

subtropical regions ***(Sivakamavalli et al., 2021)***. However, the sustainability of this industry in many countries is hindered by shrimp diseases ***(El-bouhy et al., 2006)***, in particular those caused by *Vibrios* ***(Yu et al., 2023)***. Shrimp vibriosis is a significant bacterial disease that severely impacts various shrimp species ***(Abdel-Latif et al., 2022)***. This disease is a global issue that affects all marine crustaceans, including shrimp, and is caused by bacteria from the genus *Vibrio*, which are normally found as a part of the natural bacterial flora of both wild and farmed shrimp and become opportunistic pathogens when the shrimp's natural defenses are immunosuppressed ***(Annam, 2015; El Far et al., 2015)***. *Vibrio* species are ubiquitously distributed in aquatic and marine environments ***(Baker-Austin et al., 2018)***, constituting up to 60% of the total heterotrophic bacterial population in these ecosystems ***(Annie Selva Sonia and Lipton, 2012)***. *V. parahaemolyticus* is a zoonotic pathogen found in aquatic environments that impacts both human and aquaculture animal health. It is prevalent in water and poses a significant threat to shrimp, leading to substantial economic losses in the aquaculture sector worldwide ***(Maluping et al., 2005; Maluping et al., 2008)***. This pathogen is often associated with shrimp infections due to the bacterial contamination in their environments ***(Bughe et al., 2016)***. It has also been recognized as the pathogen responsible for acute hepatopancreatic necrosis disease (AHPND) in penaeid shrimp ***(Tran et al., 2013)***, previously known as early mortality syndrome (EMS), which was first identified as a new shrimp disease in China in 2009 ***(Li et al., 2017)***. Besides the AHPND-causing *V. parahaemolyticus* strains, non-AHPND strains of this pathogen also result in serious illnesses in shrimp, leading to significant financial impacts ***(Zhang et al., 2023)***. *V. parahaemolyticus* is a halophilic, Gram-negative bacterium found in marine environments ***(Thongjun et al., 2013; Letchumanan et al., 2014; Yingkajorn et al., 2014; Ghenem et al., 2017)***. Temperature and salinity can directly affect the presence of *V. parahaemolyticus* in the environment, with its abundance increasing in warmer waters, leading to more frequent infections during the warmer months ***(Obaidat et al., 2017)***. In terms of public health threats, *V. parahaemolyticus* is believed to be a major seafood-borne pathogen affecting humans worldwide ***(Odeyemi, 2016)***. It was initially identified in 1950 as a cause of foodborne illness in Japan ***(Daniels et al., 2000)***. This bacterium accounts for up to 25% of all foodborne disease cases, surpassing other *Vibrio* species ***(Feldhusen, 2000)***. Antimicrobial resistance is thought to be a serious public health issue that is spreading

globally ***(Eid et al., 2016; Algammal et al., 2022; Shafiq et al., 2022)***. Multidrug resistant microorganisms from various sources and origins have been highlighted in a number of prior reports ***(Badawy et al., 2022; Algammal et al., 2023; Algammal et al., 2024)***. This exploratory study sought to determine the prevalence, clinical manifestations, and postmortem results of shrimp infection with *V. parahaemolyticus*.

Materials and methods:

Sampling

About 150 freshly moribund whiteleg shrimp samples (*Penaeus vannamei*) were collected in a random manner from various locations and fishermen in Ismailia Governorate. For clinical, postmortem, and bacteriological examination, samples were kept in sterile polythene bags and rapidly transferred in iceboxes to a fish diseases lab for further investigation. Clinical and postmortem examinations were carried out according to ***Austin and Austin (1989)***.

Isolation and identification of *V. parahaemolyticus* from shrimp

V. parahaemolyticus isolation was carried out according to the method described by ***Kaysner and Depaola (2004)***. For enrichment, 10 g of each pooled sample were blended in a sterile homogenizer with 90 mL of sterile alkaline peptone water and incubated at 37 ºC for 24 h. Selective plating was performed on Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar, where loopfuls samples from each enriched homogenate were streaked onto the medium and left incubated at 37 ºC for 24 h. Suspected colonies (green colonies) were purified by sub-culturing on fresh TCBS agar plates and re-incubated under the same circumstances. Pure isolates were streaked onto slanted tryptic soy agar (TSA) and then screened out for further biochemical identification using oxidase test, catalase test, TSI agar test, citrate utilization, ornithine decarboxylase, lysine decarboxylase and β-galactosidase (ONPG) according to ***(Quinn et al., 2011)***.

Results:

Clinical findings and post mortem examination of shrimp naturally infected with *V. parahaemolyticus*

Some of the naturally infected shrimp showed eroded cuticle, brownish to black patches scattered all over the body surface Figure(1), sloughing of the abdominal appendages Figure(2), dark and reddish discoloration on pleopods, periopods, carapace and tail area Figure(3). The most common postmortem findings in the moribund shrimp were whitish musculature Figure (4), and the hepatopancreas in most cases was either congested or atrophied.

Morphological characterization and prevalence of *V.*

***parahaemolyticus* among the examined shrimp samples**

Morphologically, *V. parahaemolyticus* on TCBS agar appeared as green or bluish green centered colonies (Figure 5). Microscopically, all isolates were Gram-negative, straight or slightly curved short rods. Biochemically, all the retrieved isolates were positive for oxidase, catalase, lysine decarboxylase, ornithine decarboxylase with red slant (alkaline), yellow butt (acidic) for TSI test, while, negative for the ONPG test and citrate utilization test (Table 1). *V. parahaemolyticus* was detected with a total prevalence of 14.7% (22/150) among the examined shrimp samples.

Figure (1): Naturally infected shrimp with *V. parahaemolyticus* showing erosion of the cuticle (arrow), with brownish to black patches scattered all over the body surface.

Figure (2): Naturally infected shrimp with *V. parahaemolyticus* showing destruction of the abdominal appendages (arrows).

Figure (3): Naturally infected shrimp with *V. parahaemolyticus* showing dark and reddish discoloration on the carapace, pleopods, periopods, and tail.

Figure (4): Naturally infected shrimp with *V. parahaemolyticus* showing cloudy musculature.

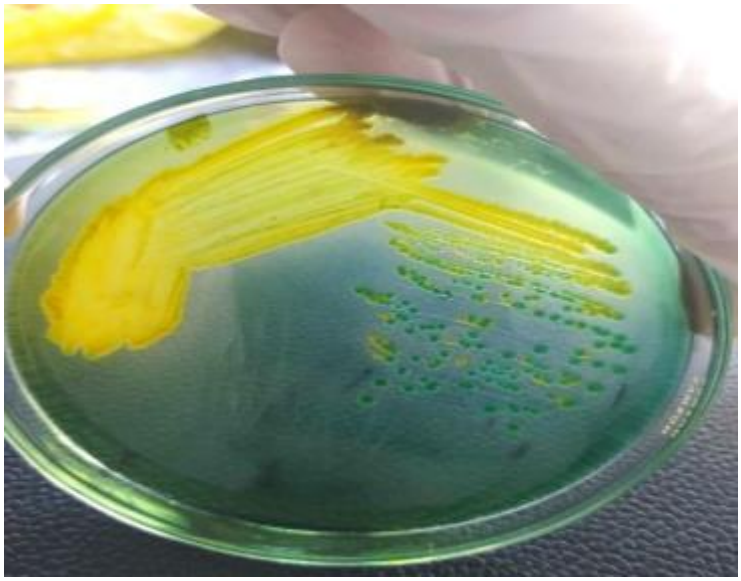

Figure (5): Green or bluish green-centered colonies of *V.parahaemolyticus* on TCBS agar.

Table 1: *Morphological and biochemical characters of V. parahaemolyticus*

Characters	***V. parahaemolyticus***
Growth on TCBS	Green/bluish green colonies
Gram's stain	Negative
Shape	Straight or slightly curved short rods
Cytochrome Oxidase	Positive
Catalase	Positive
Citrate utilization	Negative
TSI test	Alkaline slant/ Acidic butt
Lysine decarboxylase	Positive
Ornithine decarboxylase	Positive
ONPG	Negative

Discussion

Vibriosis is considered as a significant bacterial disease afflicting fish and shrimp aquaculture, resulting in substantial economic losses and elevated mortality rates across all countries engaged in aquaculture **(*Vaseeharan et al., 2008*)**. *Vibrio* species represent the predominant bacterial population within marine ecosystems and constitute a significant portion of the natural microflora in both wild and aquaculture-reread shrimp environments. Among these bacteria, pathogenic strains such as *V. parahaemolyticus* are commonly

found as part of the microbial flora associated with shrimp ***(Nakayama et al., 2006)***. While typically considered opportunistic pathogens, these bacteria can cause disease under conditions that favor their proliferation over the host organism ***(Brock, 1990)***.

Upon external examination, some of the naturally infected shrimp showed various observable symptoms, including eroded cuticle, brownish to black patches scattered all over the body surface, tail necrosis, dark and reddish discoloration on pleopods, periopods, carapace and tail area and gill fouling. The most common postmortem findings in the moribund shrimp were whitish musculature, and the hepatopancreas in most cases was either congested or atrophied. These findings were matched with those obtained by ***Khafagy et al. (2017)*** who reported black patches scattered on cuticle and body appendages, sloughing and necrosis of the antennal flagellum, periopods and pleopods, protruded and edematous eye, black gills, congested hepatopancreas and heart in naturally infected shrimp with *V. parahaemolyticus*. In addition, ***El-bouhy et al. (2006)*** reported cuticular erosion, sloughing of the appendages, brownish to black patches on exosceleton, destructed antennal flagellum, congested hepatopancreas and heart in naturally infected shrimp with vibriosis.

The current study revealed that *V. parahaemolyticus* was detected with a total prevalence of 14.7% (22/150) among the examined shrimp samples which closely approximated the findings of previous studies. ***Youssef et al. (2018)*** isolated *V. parahaemolyticus* from shrimp in the Suez Canal area with a prevalence of 15%. Similarly, ***Ibrahim et al. (2018)*** and ***Morshdy et al. (2023)*** detected *V. parahaemolyticus* in shrimp samples in Egypt at a proportion of 16%.

Conclusion

V. parahaemolyticus is a worldwide pathogen of shrimp causing great economic losses. Additional molecular methods should be explored to assess the epidemiological understanding of *V. parahaemolyticus* infection in shrimp.

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الملخص العربى

دراسة أولية لعدوى *Vibrio parahaemolyticus* في الجمبري

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عنيت الدراسة الحالية بتقصى مدى الإلصابة بميكروب الفيبريو باراهيموليتكس في الجمبري. تم جمع إجمالي 150 عينة من الجمبري بشكل عشوائي من الصيادين وأسواق الأسماك المختلفة بمحافظة الإسماعيلية بمصر، و خضعت العينات لفحوصات إكلينيكية و تشريحية وميكروبيولوجية. تم عزل وتعريف عدد 22 عزلة ميكروب الفيبريو باراهيموليتكس من العينات التي تم فحصها. أظهرالفحص الالكلينيكى تآكل بالقشرة الخارجية، وبقع بنية إلى سوداء منتشرة على جميع أنحاء سطح الجسم، وتآكل بالزوائد البطنية وتغيرًا في اللون الي اللون الداكن والمحمر على أرجل المشي و السباحة، و الدرع ومنطقة الذيل. وقد تبين من الفحص الداخلي أن بعض الجمبري اظهر عضلات بيضاء وكانت منطقة الهيباتوبنكرياس إما محتقنة في بعض الحالات أو بها ضمور في حالات أخرى. على الوسط المخصص، شكلت هذه البكتريا مستعمرات خضراء، وكان شكل هذه البكتريا عصوية الشكل وسلبية الجرام تحت الميكروسكوب. وقد خلصت الدراسة إلى تاكيد المخاطر التى يسببها ميكروب الفيبريو باراهيموليتكس والتي تهدد صحة الجمبري.