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Risk of Acquiring *Vibrio parahaemolyticus* in Water and Shrimp from an Aquaculture Farm

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Abstract

Vibrio parahaemolyticus is an important foodborne pathogen causing significant economic problems within the aquaculture industry worldwide. This study was conducted to determine the occurrence and concentration of *V. parahaemolyticus* in water and shrimp from aquaculture farms. The relationship between the concentration of *V. parahaemolyticus* in ponds and environmental parameters was also studied. A total of 264 water samples and 27 shrimp samples from a selected aquaculture farm were examined using the most probable number-polymerase chain reaction (MPN-PCR) method, a method that incorporates both the count and detection of *toxR* gene. The correlation between the concentration of *V. parahaemolyticus* in both types of the samples and environmental parameters (temperature, pH and salinity) was analyzed statistically by the paired T-test. Based on the results, 132 water samples (50%) and 11 shrimp samples (41%) were *toxR* positive. The concentration of *V. parahaemolyticus* in all the water and shrimp samples using MPN method was found to be more than 1,100 MPN/g. The environmental parameters examined were not significantly correlated with the concentration of *V. parahaemolyticus* in both types of samples. The results concluded that there was occurrence of *V. parahaemolyticus* in the aquaculture farm. Therefore, this study highlighted the potential risk of infection by *V. parahaemolyticus* from an aquaculture farm to humans. The findings obtained from this study could act as baseline data for the risk assessment of *V. parahaemolyticus* in water and shrimp samples from aquaculture farms. This will further provide useful surveillance data for relevant authorities.

Key words: Aquaculture, Shrimp, Water, MPN-PCR, *Vibrio parahaemolyticus*

1. Introduction

The gram-negative halophilic *Vibrio parahaemolyticus* is usually found in tropical and temperate coastal waters, as well as in shrimp aquaculture (Norma *et al.,* 2009). It normally causes gastroenteritis with nausea, acute watery diarrhea and abdominal cramp (Gopal *et al*., 2005). Serious infection includes septicemia that threatens the life of immunodeficient groups and prolonged steroid users (Reham and Amani, 2012). As it is one of the leading causes of foodborne outbreaks in Asian countries including Malaysia (Tunung *et al.,* 2010), it triggered our interest as an important subject for investigation. In Malaysia, the shrimp aquaculture industry has been growing steadily due to government incentives, active participation from farmers and a consistent good market price (Hashim and Kathamuthu, 2005). Nevertheless, vibriosis had resulted in mortality and serious economic loss to Malaysia in recent years (FAO, 2012).

As a consequence of increasing outbreaks by *V. parahaemolyticus*, numerous rapid methods for the detection and enumeration of this pathogen have been implemented (Blanco-Abad *et al.,* 2009). Molecular approach targeting certain genes such as toxin operon (*toxR*) gene has been reported by other researchers such as Zulkifli *et al*. (2009) and Gavilan *et al*. (2013). According to Borowsky *et al*. (2007), the quantification of microorganism present in the samples is important for assessing the risk to consumers. Recent studies by Ponniah *et al*. (2010) and Tunung *et al*. (2010) indicated that the most probable number-polymerase chain reaction (MPN-PCR) method can be used for both the qualitative and quantitative analysis of *V. parahaemolyticus*. This method also allows the simultaneous detection of *V. parahaemolyticus* in MPN tube and it is more sensitive

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than the conventional culturing method (Nordstrom *et al*., 2007). Martin *et al*. (2004) stated that the MPN-PCR method enables the completion of enumeration within two days.

The main objective of this study was to determine the occurrence and concentration of *V. parahaemolyticus* in water and shrimp samples from an aquaculture farm using the MPN-PCR method. In addition, the study aimed to investigate the relationship between the concentration of *V. parahaemolyticus* in the ponds and environmental parameters, namely temperature, pH and salinity. This study will give an insight on the current risk of *V. parahaemolyticus* from the aquaculture environment in Sarawak, which is useful in helping to control the foodborne outbreak.

2. The occurrence and concentration of *Vibrio parahaemolyticus*

A total of 264 water samples and 27 shrimp samples were collected from two ponds, designated as Pond A and Pond B from a selected aquaculture farm in Sarawak. During each sampling trip, the environmental parameters namely temperature, pH and salinity were measured using a thermometer (G H Zeal ASTM), a pH meter (pH510, Eutech Instrument) and a salinity refractometer (Hisamatsu Atago S/Mill), respectively. All the samples were pre-enriched in Alkaline Peptone Water (APW) with pH 8.5-8.6 and 1-2 %(w/v) of NaCl (Harwood *et al*., 2004). They were subjected to the three-tube MPN method. After incubation at 37 °C for 18 h, we found out that all the MPN tubes were turbid with a concentration of more than 1,100 MPN/g, indicating the occurrence of microorganisms in all the samples. They were then subjected to microbial plate count and species specific PCR analysis. The mean concentration of *V. parahaemolyticus* from Pond A and Pond B was 5.9 x 10^8 CFU/ml and 1.3 x 10^9 CFU/ml, respectively. The results analyzed using the paired T-test indicated that they were not significantly different ($P>0.700$, $p<0.05$).

DNA was extracted from the turbid MPN tubes using the boil cell method (Vengadesh *et al*., 2012). Species specific PCR was carried out to detect *toxR* gene with amplicon at 368 bp (Kim *et al*., 1999). Fig. 1 showed the gel images obtained from the specific PCR for water and shrimp samples. Out of the 264 water samples examined, 132 samples (50%) were *toxR* positive. On the other hand, 11 positive samples (41%) were detected from the total of 27 shrimp samples. The occurrence of *V. parahaemolyticus* detected using PCR on water and shrimp samples from two different ponds was summarized in Table 1. Our results were consistent with the reports by previous studies where the occurrence of *V. parahaemolyticus* in water samples was about 50% (Sujeewa *et al*., 2009) and ranged from 2.6 to 80% for shrimp samples (Zulkifli *et al*., 2009; Merwad *et al*., 2011). In brief, the turbidity in MPN tubes is an indica-

Table 1. Occurrence of *V. parahaemolyticus* in water and shrimp samples from two different ponds in an aquaculture farm.

Sample	Pond A		Pond B	
	No.[a]	%[b]	No.[a]	%[b]
Water	72/141	51	60/123	49
Shrimp	8/15	53	3/12	25

[a] Number of positive samples/number of samples examined.
[b] Occurence (in %) of positive samples among the samples examined.

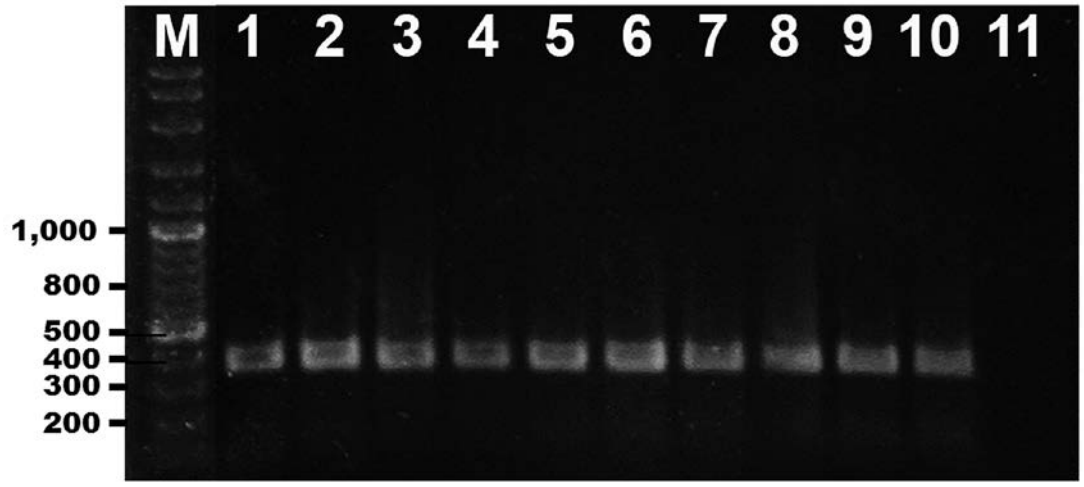

Fig. 1. Representative amplification of *toxR* gene for the identification of *V. parahaemolyticus* at 368 bp. Lane M shows the 100 bp DNA ladder, (1) positive control, (2) to (6) representative positive water samples, (7) to (10) representative positive shrimp samples and (11) negative control.

tion of the presence of a wide broad of microorganisms whereas PCR conducted in this study was specific to *V. parahaemolyticus*.

3. The relationship between concentration of *Vibrio parahaemolyticus* and environmental parameters

There was no significant difference between the concentration of *V. parahaemolyticus* in the samples and temperature as the study area has little variation in environmental temperature due to controlled temperature in both ponds. This was in agreement with the study carried out by Renata *et al*. (2010) which reported that there was no correlation between temperature and MPN value in the areas with modest temperature. On the other hand, it is a fact that Vibrio grows best in temperatures between 17 and 35 °C (Cervino *et al*., 2004). Zulkifli *et al*. (2009) also mentioned that a high marine temperature between 25 and 35 °C resulted in the distribution of *V. parahaemolyticus* all year round. As a result, it could be inferred that in this study, the occurrence of *V. parahaemolyticus* in all the samples might be attributed to the favourable environmental temperature (26 to 27 °C) of the study site.

A high pH value favours the growth of vibrios. As an example, the high pH value of the Coreaú river in Brazil was the most important environmental factor resulting in the great abundance of Vibrios in that area (Costa *et al*., 2010). Adding to this, Donovan and Netten (1996) considered pH 8.4-8.6 as an optimum pH value for the growth of *V. parahaemolyticus*. In this study, the pH value observed during sampling (pH 6.5-8.0) was not within the ideal range, yet the MPN value was more than 1,100 MPN/g. Therefore, it could be deduced that the distribution of *V. parahaemolyticus* in this aquaculture farm was not affected by the pH value.

The samples examined in this study were collected during the rainy season, which might indirectly affect the salinity of the ponds. In this study, salinity ranged from 13 to 22 ppt and it negatively correlated with the occurrence of *V. parahaemolyticus*. Our finding is not in agreement with Renata *et al*. (2010) who reported that there was no significant difference between the salinity and occurrence of *V. parahaemolyticus*. Besides, Prasanthan *et al*. (2011) stated that the salinity did not affect the growth of *V. parahaemolyticus* as this bacterium is halophilic in nature.

Lastly, the risk assessment of *V. parahaemolyticus* in water and shrimp samples from aquaculture farms is of significant importance. According to the assessment of microbiological quality set by International Committee of Microbiological Specification for Foods (ICMSF, 1986), the concentration of *V. parahaemolyticus* in Pond A and Pond B (5.9×10^8 CFU/ml and 1.3×10^9 CFU/ml, respectively) fall into the unacceptable class (Table 2), hence this poses the infection risk to human.

As a conclusion, this study demonstrated the distribution of *V. parahaemolyticus* in water and shrimp samples in a selected aquaculture farm. Since the presence of *V. parahaemolyticus* was detected in this study, a surveillance program for *V. parahaemolyticus* in aquaculture farms is therefore very important to prevent any foodborne outbreak due to the emerging pathogen.

References

Blanco-Abad V., Ansede-Bermejo J., Rodrigues-Castro A., Martinez-Urtaza J. 2009. Evaluation of different procedure for the optimized detection of *Vibrio parahaemolyticus* in mussels and environmental samples. International of Food Microbiology, 129: 229-236.

Borowsky L.M., Schmidt V., Cardoso M. 2007. Estimation of most probable number of *Salmonella* in minced pork samples. Brazilian Journal of Microbiology, 38: 544-546.

Cervino J.M., Hayes R.L., Polson S.W., Polson S.C., Goreau T.J., Martinez R.J., Smith G.W. 2004. Relationship of *Vibrio* species infection and elevated temperature to yellow blotch/band disease in Carribean corals. Applied and Environmental Microbiology, 70(11): 6855-6864.

Costa R.A., Silva G.C., Peixoto J.R.O., Vieira H.F., Vieira R.H.S.F. 2010. Quantification and distribution of vibrio species in water from an estuary

Table 2. Microbiological limits of *V. parahaemolyticus* in food (ICMSF, 1986).

Microbiological limits for assessment of microbiological quality of ready-to-eat foods				
Criterion	Microbiological quality Colony-forming unit (CFU) per gram unless specified			
	Class A Satisfactory	Class B Acceptable	Class C Unsatisfactory	Class D Unacceptable
Pathogens (applies to all food categories)				
V. parahaemolyticus	<20	20 - <100	100 - $<10^3$	$\geq 10^3$

in Ceará-Brazil impacted by shrimp farming. Brazilian Journal of Oceanography, 58(3): 183-188.

Donovan T.J., Netten P. 1995. Culture media for the isolation and enumeration of pathogenic *Vibrio* species in food and environmental samples. International Journal of Food Microbiology, 26: 77-91.

Food and Agriculture Organization. 2012. *Fishstat Plus*, Fisheries Production Database, software (in CD).

Gavilan R.G., Zamudio M.L., Martinez-Urtaza. 2013. Molecular epidemiology and genetic variation of pathogenic *Vibrio parahaemolyticus* in Peru. PLoS Neglected Tropical Diseases 7(5), e2210. doi:10.1371/journal.pntd.0002210.

Gopal S., Otta S.K., Karunasagar I., Nishibuchi M., Karunasagar I. 2005. The occurrence of *Vibrio* species in tropical shrimp culture environments; implications for food safety. International of Food Microbiology, 102: 151-159.

Harwood V.J., Ghandi J.P., Wright A.C. 2004. Method for isolation and conformation of *Vibrio vulnificus* from oyster and environmental sources. A Review of Journal of Microbiological Methodology, 59: 301-316.

Hashim M., Kathamuthu S. 2005. Shrimp farming in Malaysia. In: Regional technical consultation on the aquaculture of *P. vannamei* and other exotic shrimps in Southeast Asia, Manila, Philippines (pp. 50-56). Tigbauan, Iloilo, Philippines: EAFDEC Aquaculture Department.

International Committee of Microbiological Specification for Foods (ICMSF). 1986. *Microorganisms in Foods 2. Sampling for Microbiological Analysis: Principles and Specific Applications*, 2nd edition, University of Toronto Press, Toronto (ISBN 0-8020-5693-8).

Kim Y.B., Okuda J., Matsumoto C., Takahashi N., Hashimoto S., Nishibuchi M. 1999. Identification of *Vibrio parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. Journal of Clinical Microbiology, 37: 1173-1177.

Martin B., Jofre A., Garrigo M., Hugas M., Aymerich, T. 2004. Quantification of *Listeria monocytogenes* in fermented sausages by MPN-PCR method. Letters in Applied Microbiology, 39: 290-295.

Merwad A.M.A., El-Ghareeb W.R., Taisir S.M. 2011. Occurrence of some zoonotic vibrios in shellfish and diarrheic patients with regard to *tdh* gene in *Vibrio parahaemolyticus*. Journal of American Science, 7(9): 449-459.

Nordstrom J.L., Vickery M.C.L., Blackstone G.M., Osorio C.R., Collins M.D., Toranzo A.E., Barja J.L., Murray S.L., DePaola A. 2007. Development of multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* in oysters. Application of Environmental Microbiology, 73(18): 5840-5847.

Norma H., Irene W., Santos G. 2009. *Microbiologically safe foods*. John Wiley & Sons, Inc. New Jersey.

Prasanthan V., Udayakumar P., Sarathkumar, Ouseph P.P. 2011. Influence of abiotic environmental factors on the abundance and distribution of Vibrio species in coastal waters of Kerala, India. Indian Journal of Geo-Marine Sciences, 40(4): 587-592.

Ponniah J., Tunung R., Margaret S.P., Son R., Farinazleen Y., Malakar P.K. 2010. *Listeria monocytogenes* in raw salad vegetables sold at retail level in Malaysia. Food Control, 21: 774-778.

Reham A.A., Amani M.S. 2012. Specific detection of pathogenic *Vibrio* species in shellfish by using multiplex polymerase chain reaction. Global Veterinaria, 8(5): 525-531.

Renata A.C., Giselle C.S., Jackson R.O.P., Gustavo H.F.V., Regine H.S.F.V. 2010. Quantification and distribution of *Vibrio* species in water from an estuary in Cearà-Brazil impacted by shrimp farming. Brazillian Journal of Oceanography, 58(3): 183 -188.

Sujeewa A.K.W., Norrakiah A.S., Laina M. 2009. Prevalence of toxic genes of *Vibrio parahaemolyticus* in shrimps (*Penaeus monodon*) and culture environment. International Food Research Journal, 16: 89-95.

Tunung R., Margaret S.P., Jeyaletchumi P., Chai L.C., Zainazor T.C., Ghazali F.M., Nakaghuchi Y., Nishibuchi M., Son R. 2010. Prevalence and quantification of *Vibrio* in raw salad vegetables at retail level. Journal of Microbiology and Biotechnology, 20(2): 391-396.

Vengadesh L., Son R., Yoke-Kqueen, C. 2012. Molecular quantification and characterization of *Vibrio cholerae* from different seafood obtained from wet market and supermarket. International Food Research Journal, 19(1): 45-50.

Zulkifli Y., Alitheen N.B., Son R., Yeap S.K., Lesley M.B., Raha A.R. 2009. Identification of *Vibrio parahaemolyticus* isolates by PCR targeted to the *toxR* gene and detection of virulence genes. International Food Research Journal, 16: 289-296.