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-

![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.](image)

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pond A</th>
<th>Pond B</th>
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<tr>
<td></td>
<td>No. a</td>
<td>% b</td>
</tr>
<tr>
<td>Water</td>
<td>72/141</td>
<td>51</td>
</tr>
<tr>
<td>Shrimp</td>
<td>8/15</td>
<td>53</td>
</tr>
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a Number of positive samples/number of samples examined.
b Occurrence (in %) of positive samples among the samples examined.
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 x 10^8 CFU/ml and 1.3 x 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


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<th>Table 2. Microbiological limits of V. parahaemolyticus in food (ICMSF, 1986).</th>
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<td>Microbiological limits for assessment of microbiological quality of ready-to-eat foods</td>
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<tr>
<td>Criterion</td>
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<td>------------</td>
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<td>Pathogens (applies to all food categories)</td>
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<td>V. parahaemolyticus</td>
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