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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

PREVALENCE OF *Vibrio cholerae* IN COCKLES IN SELANGOR AND PAHANG, MALAYSIA

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Chairman: Professor Fatimah Abu Bakar, PhD

Faculty: Food Science and Technology

The aim of this study is to determine the presence of *Vibrio cholerae* in raw cockles (*Anadara granosa*) sold in wet markets in Selangor and Pahang, Malaysia and to evaluate the survival of *V. cholerae* using the polymerase chain reaction in combination with the most probable number (MPN-PCR) and the conventional method (growing on the TCBS-biochemical test). In addition, this study attempts to explore the survival of *V. cholerae* in water, during heat treatment and during the storage of raw cockles. This study is done due to *V. cholerae* is the etiological agent of cholera which is spread by contaminated food, water or attributed to raw products eaten unprocessed and also commonly found in cockles, thus increase the risk of poisoning potential due to its consumption. A total of 100 samples from 8 different wet markets in Selangor and Pahang were examined for the presence of *V. cholerae*. The prevalence of *Vibrio* spp. between the samples from two different sampling areas was not significantly different ($p>0.05$). In fact, 74% of the samples from Pahang were found positive of *Vibrio* spp. contrasting to 69% of samples from Selangor, and the prevalence of *V. cholerae* was also found to be not significantly

different between samples from Pahang, 56% and Selangor, 59%. As a comparison, 96% of samples were positive, indicating the detection of *V. cholerae* using the MPN-PCR method, while only 65% samples were positive when run under the conventional method. The results of the MPN-PCR analysis showed that the positive detection rate was high, while the results of the conventional method were always negative. With MPN-PCR, the load detected in all samples ranged from <30 up to >24000 MPN/g, but most of the samples (24 samples) contained >24000 MPN/g concentration. The enumeration of *V. cholerae* on TCBS agar was unreliable because of the problem of interference with microflora of similar morphology with *V. cholerae* that had grown in abundance on TCBS.

For the survival of *V. cholerae* in water, raw cockles (uninoculated raw cockles with *V. cholerae*) and water (distilled water) samples were determined. *V. cholerae* can transfer from infected cockles to the surrounding water. The *V. cholerae'*s load in the samples was 24000 MPN/g on the first day of incubation. The density of *V. cholerae'*s load in water environment increased from 0 MPN/g to 2400 MPN/g because the pathogens in the cockle samples were transferred to the water. The presence of *V. cholerae* in distilled water was not only sourced from meat and fluid of infected cockles but also from its shell. In the heat treatment (boiling and grilling) and storage study, two methods of analyses were used, MPN-PCR and conventional method. When boiling the cockles, the result illustrated that there was a significant difference ($p \leq 0.05$) for the four temperatures (100 °C, 90 °C, 80 °C and 70 °C) used for both analyses. Cockles need to be cooked for more than 4 minutes in 100 ºC boiling water, 5 minutes in 90 ºC of water and 6 minutes in 80° C, where the pathogen was no longer detected (<1.00 log CFU/g and <1.00 log MPN/g). When

heated at 70° C, 9 minutes is sufficient to eliminate the *V. cholerae*. In the grilling process, there was also a significant difference ($p \leq 0.05$) between three temperatures (150 ºC, 200 ºC and 250 ºC) used for both analyses. When cockles are grilled at 150 ºC, they should be cooked for 18 minutes to reduce the *V. cholerae*'s population to <1.00 log CFU/g and <1.00 MPN/g. Similarly, 12 minutes are needed to grill the cockles at 200 °C and 8 minutes at 250 ºC, where it is sufficient to eliminate the pathogen.

For the storage study, the populations of *V. cholerae* increased when cockles were stored at 10 °C and 28 °C with their shell intact (cockles' meat with shell) and samples with no shell (cockles's meat only). However, it decreased gradually when stored at 0 ºC for 16 days. During the storage at 0 ºC, the population of the *V. cholerae* in unshelled samples decreased and was significantly lower than the shelled samples for both analyses. During storage at 10 ºC for 10 days, it was observed that *V. cholerae* was able to multiply in both shell and non-shell samples and this shows that 10 ºC storage is not sufficient to inhibit the growth of *V. cholerae*. The *V. cholerae*'s load in non-shelled samples, which were stored at 28 ºC for 20 h, increased higher than the shelled samples. As the conclusion, the *V. cholerae* and *Vibrio* spp. 's loads in cockles are high and the cockles need to be boiled and grilled in sufficient time before consuming, to avoid potential poisoning. In addition, *V. cholerae* can be transferred easily from infected cockles to the surrounding water and can survive in the storage condition (chilled and ambient temperatures). The combined MPN-PCR method is more effective and accurate for the detection of *V. cholerae* over the conventional method.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

KEMANDIRIAN *Vibrio cholerae* DI DALAM KERANG MENTAH DI SELANGOR DAN PAHANG, MALAYSIA

Oleh

SUZITA BINTI RAMLI

April 2012

Pengerusi: Profesor Fatimah Abu Bakar, PhD

Fakulti: Sains danTeknologi Makanan

Tujuan penyelidikan ini adalah untuk mengenalpasti kehadiran bakteria *Vibrio cholerae* pada kerang dari pasar basah di Selangor dan Pahang, Malaysia dan menilai kemandirian dengan menggunakan kaedah gabungan MPN-PCR dan juga kaedah lazim. Selain itu juga, penyelidikan ini adalah untuk mengkaji berkaitan dengan kemandirian *V. cholerae* di dalam air, semasa pemanasan dan juga semasa penyimpanan sejukbeku. Seratus sampel dari 8 pasar basah yang berbeza di Selangor dan Pahang telah diperiksa untuk kehadiran *V. cholerae*.

Kelaziman *V. cholerae* di dua kawasan ini adalah hampir sama. 74% sampel dari pasar basah di Selangor telah didapati positif sementara 69% sampel dari pasar basah di Pahang sementara kelaziman *V. cholerae* juga hampir sama untuk kedua-dua kawasan dengan sampel dari Selangor adalah 59% dan Pahang adalah 56%. Di dalam perbandingan dua kaedah pengenalpastian, 96% adalah positif untuk pengenalpastian *V. cholerae* dengan menggunakan kaedah MPN-PCR berbanding kaedah lazim yang mengenalpasti sebannyak 65%. Dengan kaedah MPN-PCR, kadar

pengenalpastian positif sampel adalah tinggi dan had pengenalpastian adalah rendah sementara keputusan daripada kaedah lazim biasanya negatif. Dengan MPN-PCR , kepadatan patogen yang dikenalpasti pada semua sampel adalah dalam lingkungan ≤ 30 hingga ≥24000 MPN/g. Penghitungan *V. cholerae* yang dikira di atas agar TCBS adalah tidak tepat kerana masalah kehadiran mikroflora yang mempunyai morfologi yang sama dengan *V. cholerae* apabila ditumbuhkan di atas agar TCBS.

Untuk kemandirian *V. cholerae* di dalam air, kedua-dua sampel iaitu kerang (yang tidak disuntik dengan *V. cholerae*) dan air (air suling) telah dikenalpasti. Untuk sampel kerang, kepadatan *V. cholerae* adalah 24000 MPN/g untuk hari pertama. Kepadatan *V. cholerae* dalam air meningkat daripada 0 MPN/g kepada 2400 MPN/g disebabkan perpindahan patogen di dalam kerang kepada air. *V. cholerae* juga boleh didapati pada kulit kerang. Dalam pemanasan dan penyimpanan sejukbeku, dua kaedah (MPN-PCR dan kaedah konvensional) telah digunakan. Untuk pemanasan, kerang perlu dimasak lebih 4 minit di dalam 100 ºC air mendidih, dalam air bersuhu 90 ºC, populasi *V. cholerae* menurun daripada 5.53 kepada 0.16 log CFU/g dan 1.89 log MPN/g apabila dipanaskan selama 5 minit dan 30 saat, dalam air bersuhu 80 ºC, 6 minit mencukupi dan 9 minit diperlukan untuk merebus kerang dalam 70 ºC. Dalam proses memanggang, ketiga-tiga suhu digunakan (150 ºC, 200 ºC dan 250 ºC) adalah berbeza ($p<0.05$) untuk kedua-dua analisis bagi setiap masa. Dalam 150 ºC proses memanggang, kerang perlu dimasak lebih 18 minit (<1.00 log CFU/g, MPN/g). Untuk 250 ºC, 8 minit, sudah mencukupi dan 12 minit apabila memanggang pada 200 ºC. Apabila suhu tinggi digunakan, lebih singkat masa diperlukan untuk membasmi patogen dan selamat untuk dimakan. Lingkungan suhu antara 70 ºC -100 ºC, boleh digunakan untuk merebus kerang tetapi masa yang

diambil untuk membasmi patogen pada kerang mentah, berbeza berdasarkan suhu yang digunakan. Pemanggangan juga perlu dilakukan pada masa yang berbeza berdasarkan suhu yang digunakan.

Untuk penyimpanan penyejukbekuan, populasi *V. cholerae* meningkat apabila kerang disimpan pada 10 ºC dan 28 ºC untuk kedua-dua stok isi kerang dan stok kerang yang bercengkerang. Bagaimanapun, ia menurun apabila disimpan pada 0 ºC dalam masa 16 hari. Dalam penyimpanan sejukbeku pada suhu 0 ºC, populasi *V. cholerae* dalam sampel isi adalah kurang berbanding sampel bercengkerang di kedua-dua analisis. Simpanan sejukbeku pada 10 ºC dalam 10 hari, kajian mendapati *V. cholerae* masih mampu membiak di dalam kedua-dua sampel isi kerang dan sampel cengkerang dan ini membuktikan simpanan 10 ºC tidak mencukupi untuk merencat pertumbuhan *V. cholerae*. Sampel isi kerang menunjukkan kenaikan yang lebih tinggi untuk *V. cholerae* apabila disimpan pada 28 ºC untuk 20 jam berbanding sampel kerang bercengkerang. *V. cholerae* masih mampu untuk hidup di dalam stok isi kerang dan stok kerang bercengkerang apabila disimpan pada suhu rendah dan suhu bilik. Kesimpulannya, kadar kepadatan *V. cholerae* dan *Vibrio* spp. Di dalam kerang mentah adalah tinggi dan perlu direbus dan dipanggang dalam masa yang mencukupi sebelum menggunakannya untuk mengelakkan keracunan. *V. cholerae* boleh berpindah dengan mudah daripada kerang ke air di persekitarannya dan masih boleh hidup semasa penyimpanan sejukbeku. Kombinasi kaedah MPN – PCR yang digunakan membuktikan ia lebih berkesan dan tepat untuk mengenalpasti *V. cholerae* dengan penyembuhan sel yang cedera di samping mengesan kepadatan populasi *V. cholerae* berbanding pengenalpastian dengan menggunakan kaedah lazim.

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APPROVAL SHEET

I certify that a Thesis Examination Committee has met on **23 April 2012** to conduct the final examination of **Suzita Binti Ramli** on her thesis entitled **"Prevalence of *Vibrio cholerae* in Cockles in Selangor and Pahang, Malaysia"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of the type of degree. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that is has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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SUZITA BINTI RAMLI

Date:

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