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Research Article

Quantitative Risk Assessment of *Vibrio parahaemolyticus* in *Mytilus edulis* in China

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Abstract: The objective of this study was to conduct *Vibrio parahaemolyticus* risk assessment associated with the consumption of *Mytilus edulis* among the people of Zhejiang Province, China. It was based on the quantitative risk assessment framework developed by the Codex Alimentarius Commission. In order to calculate the quantitative risk assessment, the data generated in this study as well as data gathered from other reports were taken into account. The exposure assessment of *Vp* was conducted with estimation of its density in *Mytilus edulis* by using Monte Carlo simulation and considering cooking and consumption modes. The Matlab software package, version 7.10.0.499 (R2010a) in combination with Microsoft-Excel was used to run the Monte Carlo simulations. We found that the probability of illness for people eating *Mytilus edulis* per meal was 8.4×10^{-7}, which means the incidence rate of illness/10,000,000 population/year is 8.4 if each person eats 10 meals of *Mytilus edulis* in summer.

Keywords: Monte carlo simulation, *Mytilus edulis*, quantitative risk assessment, *Vibrio parahaemolyticus*

INTRODUCTION

Vibrio parahaemolyticus (*Vp*) is a Gram-negative halophilic bacterium that occurs naturally in estuarine, marine and coastal environments throughout the world (Joseph *et al*., 1982). Consumption of food contaminated with *Vp* may lead to development of acute gastroenteritis characterized by diarrhea, headache, vomiting, nausea, abdominal pain and low-grade fever (Su and Liu, 2007). With the widespread consumption of seafood, this bacterium has accounted for most cases of food poisoning in Japan, North America and Southeast Asia (Daniels *et al*., 2000). In China, during the past two decades, *Vp* has been the most common cause of the bacterial food-borne outbreaks (Wu *et al*., 2014).

The last three decades have witnessed an increase in the content of *Vp* in China seas mainly due to offshore pollution, global warming and many other factors. *Vp* is one of the major seafood-borne gastroenteritis causing bacteria and is frequently isolated from shellfish samples (Zhao *et al*., 2011). In China, the annual output of shellfish aquaculture is nearly 1 million tons, in which the *Mytilus edulis* is one of the most important commodities and Zhejiang Province is one of the main producing regions. *M. edulis* is a filter feeder, which can enrich *Vp* from its surrounding environment, so that it becomes an important carrier of *Vp* and its affiliated diseases like diarrhea. The issue of high *Vp* concentration in *M. edulis* and its high consumption in China is becoming a serious threat to public health. Therefore, there is a dire need to carry out the complete risk assessment of *Vp* so that appropriate steps could be taken to halt the spread of this pathogen via over consumption of contaminated *M. edulis*.

According to WHO, risk assessment is the scientific evaluation of known or potential adverse health effects resulting from human exposure to food-borne hazards. The process consists of the four steps: hazard identification, hazard characterization, exposure assessment and risk characterization. The definition includes quantitative risk assessment, which emphasizes reliance on numerical expressions of risk and also qualitative expressions of risk, as well as an indication of the attendant uncertainties (Sani *et al*., 2013).

The scope of this study was to assess the risk of acquiring gastroenteritis due to *Vp* for among Chinese population as a result of the consumption of *M. edulis*.

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This research will provide some basic information for the government to address the food safety public events, or may be helpful for future research.

MATERIALS AND METHODS

The detection of *Vp* in *M. edulis* was referred to SN 0173-2010《The Detection of VP in export food》. The Matlab software package, version 7.10.0.499 (R2010a) in combination with Microsoft-Excel was used to run the Monte Carlo simulations. A quantitative risk assessment of *Vp* was conducted in order to estimate the risk of getting infected by consuming *M. edulis* among the people living in the Zhoushan Islands of Zhejiang Province, China. To develop the model, the four steps of risk assessment as mentioned earlier were included: hazard identification, hazard characterization, exposure assessment and risk characterization (Sen *et al*., 2005). This risk assessment is a quantitative analysis in which the key steps from post-harvest handling to consumption were modeled. The likelihood of illness following exposure to the pathogenic *Vp* from the consumption of *M. edulis* was calculated.

Hazard identification of *Vp*: Hazard identification as a part of microbial risk assessment is the identification of known or potential pathogenic microorganism which could cause adverse health effects and is present in a particular food or group of foods. In this risk assessment, the hazard was focused on pathogenic *Vp* in *M. edulis* inadequately cooked. *Vp* infection is usually caused by eating such mussels.

Hazard characterization of *Vp*: Hazard characterization is the qualitative and/or quantitative evaluation of the nature of the adverse effects associated with a particular agent which may be present in food. For biological agents, a dose-response assessment should be performed if the data are obtainable. In this study, the section focuses on evaluating the nature of adverse health effects associated with the presence of *Vp* in *M. edulis* and how to quantitatively assess the relationship between the magnitude of the food-borne exposure and the likelihood of adverse effects occurring, providing sufficient information to allow for a quantitative measurement of the public health risk from *Vp* associated with the consumption of *M. edulis*.

Exposure assessment of *Vp*: The exposure assessment component of a microbial risk is an evaluation of the likelihood of ingesting pathogenic microorganisms via food and the likely level of exposure in a given population. In the assessment, the magnitude of exposure to *Vp* from the consumption of inadequately cooked *M. edulis* was evaluated and a mathematical model was developed to quantify the magnitude of exposure assessment for *Vp*.

Risk characterization of *Vp*: In the risk characterization, the estimated exposure is integrated with the dose-response model and consideration of cooking & consumption mode and pathogenic proportion of *Vp in M. edulis* to provide a quantified risk estimation and probability of illness for people eating *M. edulis* per meal. All calculations were performed by the Monte Carlo simulation of sampling from specified input distributions and appropriately combining the sampled values to generate the corresponding output distributions.

RESULTS AND DISCUSSION

Hazard identification of *Vp*: There are many pathogenic factors associated with *Vp*, such as the invasiveness and hematotoxin and the production of Thermostable Direct Hemolysin (TDH) is the most important, which is a reliable feature to distinguish between pathogenic and non-pathogenic strains of *Vp* (Chen and Liu, 2006). The food contaminated with *Vp* can potentially cause gastroenteritis, headache, nausea and vomiting, stomach cramps or fever and a few cases can lead to sepsis, which leads to death (Honda *et al*., 2008). The main symptoms are diarrhea, vomiting, headache, nausea, abdominal cramps, fever etc. (Dai and Song, 2005). The course is generally 2~4 d, shorter duration and self-healing (Su and Liu, 2007). Severe disease may cause wound infection and septicemia and some body tissues will change, such as bullous skin damage, adjuvant arthritis, arthritis deformans etc. (Morris and Black, 1985).

According to the monitoring data of the national food-borne disease surveillance network in China, food poisoning caused by *Vp* had occupied an important position in the bacterial food poisoning. In the food poisoning statistics from 1992~2004, the average incidence and people affected by microbial food poisoning accounted for 54.63% and 67.24%, respectively and *Vp* accounted for 33.52% of total microbial food poisoning events (Yu, 2005). The analysis of the pathogenic factors of major food poisoning in Ningbo, Zhejiang Province indicated that *Vp* accounted for 25.33% (Qin and Liu, 2004). In Zhejiang Province, in all food poisoning cases diagnosed in 2001~2014, 29% were related to *Vp* poisoning, according to an unpublished recent statistics.

Hazard characterization of *Vp*: Hazard characterization is also called dose-response, which is a quantitative description of the dose-response relationship between the concentration of pathogen and the degree of disease risk. The commonly used dose-response model for risk assessment of *Vp* are the Gompertz model, Probit model and Beta-poisson model. The former two are linear models whose index

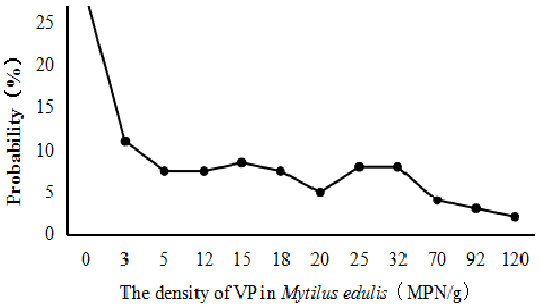

Fig. 1: Test result of *Mytilus edulis* sample from Zhoushan, Zhejiang Province, China

is a linear function of the infection dose and with bigger uncertainty compared to Beta-poisson model. In the case of considering the convenience and linear range of the model, we chose the Beta-poisson model (Yamamoto *et al*., 2008). The formula is as follows:

$$P_r(ill/d) = 1-(1+d/\beta)^{-\alpha}$$

where,

d = The dose of pathogenic *Vp*

$P_r(ill/d)$ = The probability of gastroenteritis caused by pathogenic *Vp*

α = 0.6

β = 1.3×10^6

Exposure assessment of *Vp*:

The relevance ratio of *Vp* in *Mytilus edulis*: We took *M. edulis* samples from Zhoushan sea area in Zhejiang Province of China and then detected the relevance ratio of *Vp* in *M. edulis*. The result is shown in Fig. 1. It can be used in the prediction of the growth of *M. edulis* from the sampling point to the retail point.

Prediction of the growth of *Mytilus edulis* from the sampling point to the retail point: In this study, after the *M. edulis* samples collected from the sampling point, they were refrigerated at 4°C. Result of a previous study shows that the body temperature of a single oyster in the cold storage environment (4°C) decreases exponentially and its growth rate reduces down to 0 when its body attains the temperature of 4°C (Cook, 1994). *M. edulis* and oysters are both bivalve shellfish, so we could reasonably assumed that the growth rate of *Vp* in *M. edulis* is similar to that of oysters. Data obtained from other refrigerated foods showed that the body temperature of mussels dropped to 4°C was no longer than 10 h. Through a lot of practical investigations, the total amount of VP grown in k hours was:

$$\sum_{i=1}^{k}\mu_m\times\frac{(k+1)-i}{k}=\mu_m\times[(k+1)-\frac{1}{k}\sum_{i=1}^{k}i=\mu_m\times[(k+1)-\frac{k+1}{2}]=\mu_m\times\frac{k+1}{2}$$

Table 1: VP growth rate in mussels at the temperature

Temperature (°C)	The growth rate of VP in mussels (MPN/g·h)
9	0.266185
10	0.276794
11	0.290548
12	0.307872
13	0.329315
14	0.355584
15	0.387579
16	0.426448
17	0.473651
18	0.531048
19	0.60102
20	0.686624
21	0.791798
22	0.921642
23	1.082785
24	1.283878
25	1.536240
26	1.854717
27	2.258769
28	2.773831
29	3.432888
30	4.278072
31	5.361717
32	6.745627
33	8.495929
34	10.66867
35	13.27820
36	16.23811
37	19.26930
38	21.79648
39	22.92269

The growth rate of *Vp* (μ_m) depends on the temperature of mussels before refrigeration. At different temperatures, the growth rate of *Vp* in mussels was shown in Table 1 (Li *et al*., 2012).

The density of *Vp* in *M. edulis* from the sampling point, the temperature related to the growth rate of *Vp* (μ_m) and the growth time of *Vp* (k) were all sampled randomly within the specific limits. All of the calculations were performed by the Monte Carlo simulation with 500 input times. The probability distribution was shown in the Fig. 2.

The figure shows that the density of *Vp* in *M. edulis* by prediction ranges from 0 to 130 at retail, the average is about 28 MPN/g.

Risk characterization of *Vp*: According to normal consumption mode, we assumed that the average number of *M. edulis* eaten per meal was 10.0. An average weight of shelled *M. edulis* is 6.5 g, so the average intake of *M. edulis* per meal is 65.00 g.

Intake of pathogenic *Vp* = the predicted density of *Vp* at retail × the consumption of *M. edulis* per meal × the proportion of pathogenic *Vp* (assumed 1%) × the proportion of inadequately cooked (assumed 10%).

The dose of pathogenic *Vp* per meal/person = $28\times6.5\times10\times1\%\times10\% = 1.82$

$$P_r(ill|d)=1-[1+d/(1.3\times10^6)]^{-0.6}=1-[1+1.82/(1.3\times10^6)]^{-0.6}=8.4\times10^{-7}$$

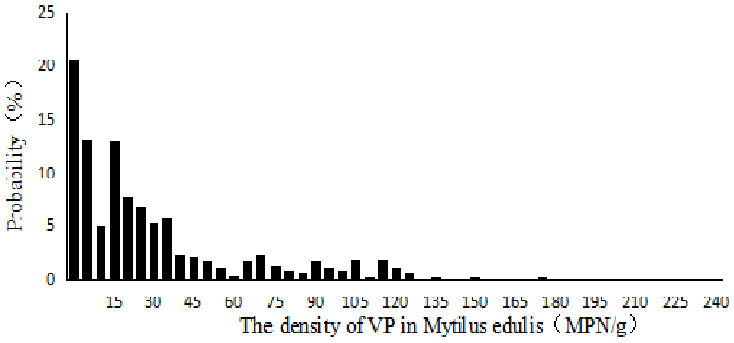

Fig. 2: Predicted VP density in *Mytilus edulis* at retail

Combining the predicted *Vp* density in *M. edulis* at retail with dose-response model, it could be concluded that the probability of illness for eating *M. edulis* per meal was 8.4×10^{-7}, which means the incidence rate of illness/10,000,000 population/year is 8.4 if each person eats 10 meals of *M. edulis* in summer.

CONCLUSION

In this study, the quantitative risk assessment procedure was set up for *Vp* in case of *M. edulis* consumption. We sequentially considered all steps of its consumption starting from initial harvest and transportation to cooking and consumption. Each step provided reasonable data or assumptions, thus the number of people that could possibly be affected by *Vp* infections was concretely calculated.

The ubiquitous nature of *Vibrio* species in marine and estuarine environments makes it impossible to obtain seafood completely free of these bacteria. Concerning its risk, though complete elimination is impossible, reduction is possible if proper procedures implemented. It is important to keep the temperature low during postharvest transportation to keep its growth rate at a minimal rate and ensure adequate cooking to kill all contaminants. The limitations of this study were that the data gathered were very sparse since they were collected randomly in a selected area. More data are needed from other areas in the future for modifying and enriching the risk assessment of *Vp*.

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REFERENCES

Chen, Y. and X.M. Liu, 2006. Quantitative risk assessment of *Vibrio parahaemolyticus* in retail raw oysters in Fujian. Chin. J. Food Hyg., 18: 103-108.

Cook, D.W., 1994. Effect of time and temperature on multiplication of *Vibrio vulnificus* in postharvest gulf coast shellstock oysters. Appl. Environ. Microb., 60(9): 3483-3484.

Dai, L. and B.Y. Song, 2005. Beware of food poisoning by eating seafood. Chin. Insp. Quarantine, 11: 61-61.

Daniels, N.A., B. Ray, A. Easton, N. Marano, E. Kahn, A.L. McShan, L. Del Rosario, T. Baldwin, M.A. Kingsley, N.D. Puhr, J.G. Wells and F.J. Angulo, 2000. Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters: A prevention quandary. JAMA, 284(12): 1541-1545.

Honda, T., T. Iida, Y. Akeda and T. Kodama, 2008. Sixty years of *Vibrio parahaemolyticus* research. Microbe, 3: 462-466.

Joseph, S.W., R.R. Colwell and J.B. Kaper, 1982. *Vibrio parahaemolyticus* and related halophilic *Vibrios*. Crit. Rev. Microbiol., 10(1): 77-124.

Li, S.S., Y.J. Wu, Q. Ning, W.Z. Wu and C.J. Fang, 2012. Quantitative risk assessment of *Vibrio parahaemolyticus* in short necked clam. J. Beijing Tech. Bus. Univ., Nat. Sci. Edn., 5: 38-45.

Morris, J.G. and R.E. Black, 1985. Cholera and other *Vibrioses* in the United States. New Engl. J. Med., 312: 343-350.

Qin, P.Z. and Y. Liu, 2004. Analysis of major food poisoning cases in Ningbo city from 1986 to 2003. Chin. Prev. Med., 5: 452-453.

Sani, N.A., S. Ariyawansa, A.S. Babji and J.K. Hashim, 2013. The risk assessment of *Vibrio parahaemolyticus* in cooked black tiger shrimps (*Penaeus monodon*) in Malaysia. Food Control, 31(2): 546-552.

Sen, S.S., V.P. Rives, O.D. Messina, J. Morales-Torres, G. Riera, J.M. Angulo-Solimano, J.F.M. Neto, A. Frisoli, Jr., R.C. Sáenz, O. Geling and P.D. Ross, 2005. A risk assessment tool (osteorisk) for identifying Latin American women with osteoporosis. J. Gen. Intern. Med., 20(3): 245-250.

Su, Y.C. and C. Liu, 2007. *Vibrio parahaemolyticus*: A concern of seafood safety. Food Microbiol., 24(6): 549-558.

Wu, Y., J. Wen, Y. Ma, X. Ma and Y. Chen, 2014. Epidemiology of foodborne disease outbreaks caused by *Vibrio parahaemolyticus*, China, 2003-2008. Food Control, 46: 197-202.

Yamamoto, A., J. Iwahori, V. Vuddhakul, W. Charernjiratragul, D. Vose, K. Osaka, M. Shigematsu, H. Toyofuku, S. Yamamoto, M. Nishibuchi and F. Kasuga, 2008. Quantitative modeling for risk assessment of *Vibrio parahaemolyticus* in bloody clams in Southern Thailand. Int. J. Food Microbiol., 124(1): 70-78.

Yu, T., 2005. An analysis on food poisoning in Zhejiang Province from 1992 to 2004. Zhejiang J. Prev. Med., 17: 26-27.

Zhao, F., D.Q. Zhou, H.H. Cao, L.P. Ma and Y.H. Jiang, 2011. Distribution, serological and molecular characterization of *Vibrio parahaemolyticus* from shellfish in the eastern coast of China. Food Control, 22(7): 1095-1100.