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A Risk Assessment of *Vibrio parahaemolyticus* for Consumption of Shucked Raw Oyster in Korea

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ABSTRACT - To assess the risk of *V. parahaemolyticus* infection caused by consumption of raw oysters in Korea, contamination levels during the retail-to-table route of oysters was modeled to predict *V. parahaemolyticus* growth based on temperature and time. The consumed amount data of the KNHANES and the standard recipe of RDA were applied. A consumption scenario for exposure assessment was developed and combined with a Beta-Poisson dose-response model. The estimated probability of illness from consumption of pathogenic *V. parahaemolyticus* in raw oysters during three separate months (April, October, and November) was 5.71×10^{-5} (within the 5th and 95th percentile ranges of 2.71×10^{-8} to 1.03×10^{-4}).The results of the quantitative microbial-risk assessment indicated that the major factors affecting the probability of illness were the initial contamination level at the retailer, the consumed amount, the prevalence of pathogenic strains [*tdh* or *trh* genes], and exposure temperature and time.

Key words : Quantitative microbial risk assessment, Raw oyster, *Vibrio parahaemolyticus*, Korea

Vibrio parahameolyticus is closely related to seafood safety. *V. parahaemolyticus* is a Gram-negative, mesophilic and halophilic foodborne pathogen that causes gastroenteritis, with symptoms including severe stomach cramps and diarrhea. Raw fish and shellfish are the most common sources of illness caused by *V. parahaemolyticus*, in addition to contaminated utensil materials or improper storage. The incidence and severity of *V. parahaemolyticus*-associated gastroenteritis are influenced by the dose of bacteria and virulence characteristics of isolated strains[1,2]. The major virulence factors of *V. parahaemolyticus* are *thermo-stable direct hemolysin* (*tdh*) and *tdh-related hemolysin* (*trh*) genes[3]. *V. parahaemolyticus* is the main foodborne pathogen in Asia including Korea[4].

The Codex Alimentarius Commission recommended that a quantitative microbial risk assessment be carried out based on scientific information, including hazard identification, exposure assessment, hazard characterization (dose-response relationship), and risk characterization[5]. A risk assessment of *V. parahaemolyticus* in raw oysters from the Pacific and Atlantic oceans was previously carried out in the U.S. in 2005[6]. However, we previously showed that contamination levels, processing methods, and consumption patterns differ between the U.S. and Korea[7,8]. To estimate the extent of exposure to pathogen due to food intake, risk factors specific to a country or region such as pathogen contamination rate and levels, eating habits, consumer behaviors during preparation, cooking method, and consumed amounts must be considered[9].

In Korea, raw oyster meat is typically distributed without the shell. Oyster shucking is carried out in coastal harvest areas, with some steps of the shucking process for domestic distribution taking place at ambient temperature. Accordingly, shucked oysters are the most useful shellfish model for predicting the worst possible outcome in terms of the safety of oyster consumption in Korea. Koreans normally consume raw oysters from October to April of the following year. During this period, *V. parahaemolyticus* outbreaks mostly occur in October, November, and April[4]. Raw oyster is consumed in a variety of ways in Korea, including as seasoned oyster or in mixed dishes with vegetables and meats; calculating the amount of raw oyster consumed is therefore complicated and requires a standard recipe.

To this end, the present study investigated the consumption patterns of Koreans in order to establish a model for raw oyster consumption that can be used assess the risk

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of *V. parahaemolyticus*-related illness for Korean consumers. We also propose a risk management plan to reduce the risk associated with *V. parahaemolyticus* in Korea.

Materials and Methods

Assumption for risk assessment

For risk assessment, the simplest probable retail-to-table pathway of raw oyster meat from its source to Korean consumers was determined (Fig. 1). The model incorporated initial contamination at the retailer and projected growth based on time and temperature during transportation prior to consumption. The risk during the months of December through March was excluded because *V. parahaemolyticus* has not been detected during this period in microbial analysis studies[7,11-13]. In addition, the risk from May to September was excluded because Koreans do not eat raw oysters during this period.

The following assumptions were made in this study due to the lack of data: 1) *tdh*- and *trh*-positive pathogenic *V. parahaemolyticus* have similar growth rates; 2) similar amounts of raw oyster are consumed in April, October, and November; 3) consumption patterns of the surveyed group are representative of the Korean population; 4) oyster temperature is the ambient temperature during transportation; 5) there is no reduction in *V. parahaemolyticus* due to consumer behaviors such as oyster washing or mixing with other ingredients during foot preparation; and 6) raw oysters were consumed immediately upon purchase by the consumer.

Exposure assessment: Collection of microbiological data on the prevalence and pathogenicity of *V. parahaemolyticus* in raw oyster

There are not many released quantitative data of *V. parahaemolyticus* in seafood requiring MPN methods with identification of pathogenic strains by PCR methods[14]. Especially quantification data of *V. parahaemolyticus* in retail oysters are limited in Korea[7,11]. Collection of quantitative monitoring data were restricted to April, October, and November in this work, since these are the most common periods of *V. parahaemolyticus* outbreak, *V. parahaemolyticus* detection in raw oysters, and raw oyster consumption in

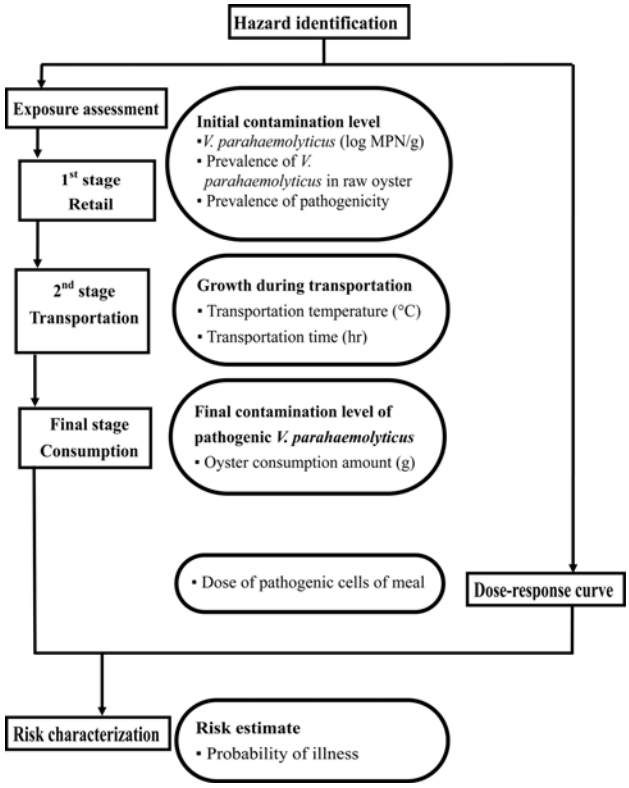

Fig. 1. Risk assessment pathways and parameters of pathogenic *V. parahaemolyticus* for raw oyster consumption in Korea.

Table 1. Number of contaminated samples and *V. parahaemolyticus* levels in raw oysters at the retail market in April, October, and November and presence of pathogenic *V. parahaemolyticus* strain ratios in seafood in Korea

log MPN/g	April	October	November	Total	Data source
ND (< −0.9)	12	1	10	23	Reference 7, 11
−0.5 < x ≤ 0	0	4	2	6	
0 < x ≤ 1	1	2	0	3	
1 < x ≤ 2		5		5	
Detection (%)	1/13 (7.7%)	11/12 (91.7%)	2/12 (16.7%)	14/37 (37.8%)	

	Place (Year)	Virulence gene	Shellfish (N)	Positive samples (%)	Data sources
Virulence data	Korea (Apr. 2005-Dec. 2005)	*tdh* or *trh*	Oysters (72)	1 (1.4%)	Reference 7
	Korea (Jan. 2000-Aug. 2001)	trh	Fish (16), shellfish (32), mollusks (24), crustaceans (11),	2 (3.6%)	Reference 15
	Korea (Jul. 2014-Oct. 2015)	*trh* data from isolated strains	oysters (96)	11(9.6%)	Reference 13

Korea (Table 1). The two studies[7,11] also supported this period risk model, as *V. parahaemolyticus* was detected from April to November but not in December through March. The *V. parahaemolyticus* contamination rate (14/37 samples, 37.8%) was higher in October (91.7%) than in November (16.7%) or April (7.7%) (Table 1). A beta distribution (r + 1, n – r + 1) was used to model the prevalence of *V. parahaemolyticus*, where n (trial number) and r (success number) were 37 and 14, respectively, and modelled as Beta (15, 24). Previous microbial analyses[12,13] of raw oysters were not included in the retail-to-table model since in those studies, oyster samples were collected at the site without retail shucking process.

Best fitting distribution of the [log most probable number (MPN)/g] of *V. parahaemolyticus* contamination level of in raw oyster (Table 1) was applied by @Risk v.6.0 software with Chi-square, A-D and K-S statistics and was shown to be Lognormal (3.066, 0.922).

Data on the virulence of *V. parahaemolyticus* isolated from oysters and seafood in the Pacific region of Korea were obtained from three studies[7,13,15] (Table 1); two of the datasets were combined to determine the presence (%) of the virulence factor among 155 samples and the number of samples positive for the virulence factor (n = 3)[7,15]. In one study, 11 pathogenic *trh* strains were isolated from a total of 115[13]. Data from the three studies were used for modeling and a beta model (15, 257) was used to establish virulence.

Exposure assessment: Model of *V. parahaemolyticus* growth according to temperature and time variables

A predictive model for temperature variables were used to predict *V. parahaemolyticus* growth at different steps along the pathway (Fig. 1). A previous study comparing the growth patterns of *trh*-positive pathogenic *V. parahaemolyticus* and non-pathogenic strains isolated from oysters showed that the former grew more slowly than the latter[16]. Since gastrointestinal illness is caused by consumption of pathogenic strains, we modeled the growth of *trh*-positive *V. parahaemolyticus* per serving of oysters during transportation using the following primary growth model of cells grown in nutrient broth with 3% NaCl:

$$Yt = No + Cmax \times \exp\,[-\exp\,([2.718 \times SGRt/Cmax] \times [LT - t] + 1)] \quad (1)$$

where Yt is the log count (cfu ml^{-1}) at time t, No is the initial level of bacteria, Cmax is the growth from inoculum to stationary phase, SGRt is relative growth rate, and t is time. A Cmax value of 9.0 from the 15°C growth curve was used in this study. The values of lag time (Lt) and specific growth rate (SGRt) of pathogenic strains as a function of temperature[16] were obtained by equations 2 and 3.

$$SGRt = [0.00219\ (T - 6.128)]^2 \quad (2)$$

$$Lt = 90.35 + (-4049/T) + (45493/T^2) \quad (3)$$

The average daily temperatures for April, October, and November in Korea[17] were 7.9°C ~ 29.3°C (the most likely temp was 20.1°C), and modelled as Pert (7.9, 20.1, 29.3). Changes in log MPN/g of *V. parahaemolyticus* during retail, transportation, and consumption of oysters were modeled based on temperature and time variables (Fig. 1).

Exposure assessment: Consumer survey for determination of transport time of raw oysters

Data on transport time of raw oysters after purchase were obtained through a consumer survey[18]. The variability in transport time (t_t) was modeled as Uniform (0.144, 4.68) using average transport time (0.006-0.195 days or 0.144-4.68 h).

Exposure assessment: Analysis of data on amount of raw oyster consumed

The amount of raw oysters consumed was calculated using Korea National Health and Nutrition Examination Survey (KNHANES) data conducted in April[19] and Rural Development Administration (RDA) standard recipe information[19]. This survey of 9,047 subjects over 12 years of age was carried out and it contains information regarding food items and amount of food consumed by respondents during 24 retrospective hours per serving per person. To account for variations in consumption caused by the cooking method, the amount of oyster (g) containing other food materials such as vegetables and meat that was consumed was calculated by multiplying the consumed amount of raw oyster-containing food (g) from the KNHANES data by the raw oyster ratio of the standard RDA recipe (Table 2). The ratio of oysters in the food was calculated from the standard RDA recipe for each person?i.e., raw oyster = 100%, seasoned oyster = 71.4%, oyster jotgal = 44.5%, seasoned oyster with radish slice = 18.2%, bossam kimchi = 2.3%, and bossam = 1.5% (data not shown). The consumed amount (Ca) of each food portion containing raw oysters (KNHANES data) was multiplied by the raw oyster ratio (RDA data of standard recipe) to calculate the amount of raw oyster (g) per serving.

Best fitting distribution of the consumed amount of raw oyster (Table 2) was applied and was shown to be Exponential (8.248). *V. parahaemolyticus* exposure levels in humans (expressed as log MPN/serving) was used to calculate the amount per serving by multiplying by the final con-

Table 2. Raw oysters consumed per serving as determined by a KNHANES study and RDA standard recipe

Amount (g)	Number of servings	Percentage (%)
$0 < x \leq 5$	144	70.9
$5 < x \leq 10$	28	13.8
$10 < x \leq 20$	17	8.4
$20 < x \leq 30$	2	1.0
$30 < x \leq 40$	2	1.0
$40 < x \leq 50$	4	2.0
$50 < x \leq 100$	5	2.5
$100 < x$	1	0.5
Total number of servings	203	100

tamination level in log MPN/g of *V. parahaemolyticus* (Table 2).

Dose-response relationship

A beta-Poisson dose-response curve[21,22] was used to estimate the risk of illness caused by pathogenic *V. parahaemolyticus* in raw oysters:

$$P(ill|d) = 1 - (1 + d/\beta)^{-\alpha} \quad (4)$$

where d is dose and P(ill|d) is the probability of illness due to the pathogenic strain. When the conditions $\beta >> \alpha$ and $\beta >> 1$ were satisfied, The beta-Poisson distribution was determined to be suitable for modeling the *V. parahaemolyticus* dose-response relationship[23].

Simulations

The input parameters for *V. parahaemolyticus* risk assessment are shown in Table 3. @Risk v.6.0 software was used for Monte Carlo simulation with 10,000 iterations.

Risk characterization

The dose-response model was combined with the output of exposure assessment to estimate the probability of exposure to pathogenic *V. parahaemolyticus* per serving of

Table 3. Summary of variables, distributions, models, equations, and references used in this study

Variable	Definition	Units	Model/distribution/equation	Reference
Pv.p	Prevalence of V.p in raw oyster	-	Beta (15, 24)	Table 1
Lv.p	Level of V.p in raw oyster at retail	log MPN/g	Lognormal (3.066, 0.922)	Table 1
Ppatho	Prevalence of pathogenic genes (*tdh* or *trh*)	-	Beta (14,257)	Table 1
Ppv.p	Probability of oyster contamination by pathogenic *V. parahaemolyticus*	-	PV.p × Ppatho	
Tt	Transport temperature	°C	Pert (7.9, 20.1, 29.3)	Reference 17
tt	Transport time	h	Uniform (0.144, 4.68)	Reference 18
Ca	Consumption amount	g	Exponential (8.248)	Table 2
SGRt	Specific growth rate during transportation	-	$SGRt = 0.00219 (Tt - 6.128)^2$	Equation 2
Ltt	Lag time during transportation	h	$Ltt = 90.35 + (-4049/Tt) + (45493/Tt^2)$	Equation 3
Cmax	Cmax (maximum population density at 15°C)	log MPN/g	Fixed to 9.0	Reference 16
Gt	Growth during transportation	log MPN/g	Yt = No + Cmax × exp [−exp([2.718 × SGRt/Cmax] × [LT − t] + 1)]	Equation 1
d	Dose of pathogenic V.p in consumed meal	cells	d = power (10, Gt) × Ca	Reference 6
Pill	Probability of illness in dose-response curve of pathogenic strain	-	$P(ill|d) = 1 - (1 + d/\beta) - \alpha$	Reference 21; Reference 22

Table 4. Risk estimates

Name	Description	5th Percentile	Mean value	95th Percentile
Lv.p	Population density at retail (log MPN/g)	−0.77	0.49	2.18
Gt	Population density at consumption (log MPN/g)	−0.29	1.00	2.71
Pill	Probability of illness by consuming a meal	2.71×10^{-8}	5.71×10^{-5}	10.3×10^{-4}

raw oyster meat. A sensitivity analysis was carried out to compare input factors contributing to risk.

Results and Discussion

To predict the risk by consumption of raw oyster contaminated by pathogenic *V. parahaemolyticus* in Korea, input and output variables were described and summarized in Table 3. The input values of Lv.p (Level of V.p in raw oyster at retail), Ppatho (Prevalence of pathogenic genes (*tdh* or *trh*)), Tt (Transport temperature), tt (Transport time) and Ca (Consumption amount) were used estimate the output values of Gt (Cell population density at consumption), d (Dose of pathogenic V.p in consumed meal), and Pill (Probability of illness) (Table 3).

By Monte Carlo Simulations, the estimated mean and 5th and 95th percentile distributions of output values are shown in Table 4. According to the retail-to table model, retail oysters were contaminated with *V. parahaemolyticus* at a level of 0.49 log MPN/g (the mean value). Approximately 93.5% retail oyster in this season was estimated to be less than 2 log MPN/g by simulation (data not shown). When the oysters were transported to the location of consumption at ambient temperature, the concentration of *V. parahaemolyticus* was estimated to be 1.00 log MPN/g (the mean value) (Table 4). Given the exposure to ambient temperatures during transportation, *V. parahaemolyticus* growth was only 0.5 log MPN/g, although growth in nutrient-rich broth may be overestimated. The risk in the range of 5th to 95th percentiles ($2.71 \times 10^{-8} \sim 1.03 \times 10^{-4}$) emphasizes the worst case of contamination level, consumption amount, the presence of virulence factor, etc. The predicted mean risk of illness for oyster consumers caused by pathogenic *V. parahaemolyticus* per serving of raw oyster (Pill) was 5.71×10^{-5}, which was comparable to the mean risk determined in Brazil (4.7×10^{-4} in spring and autumn) and the Gulf Coast of the U.S. (1.7×10^{-4} and 4.3×10^{-5} in spring and autumn, respectively)[24]. Since the consumer survey by KNHANES was conducted in April, only 1.9% of the respondents were categorized as for the oyster consumer.

Data gaps were found as the prevalence of oyster consumers, limited sample size, consumer behaviors during preparation in this study. In addition, some of the box package containing ice prohibits the growth of *V. parahaemolyticus* during transportation, the risk can be reduced in this case.

The major factors influencing risk estimates for subjects consuming raw oyster in the sensitivity analysis were initial concentration of *V. parahaemolyticus* in retail oysters in log MPN per g (Lv.p); amount of raw oyster consumed (Ca); prevalence of pathogenic strains (i.e., those harboring *tdh* or *trh*); prevalence of *V. parahaemolyticus* in oysters (Pv.p); and exposure temperature and time (t) (Fig. 2). These are the major points to reduce the risk. The variation in risk due to different pathogenic strains requires further investigation. One study reported a case of *Vibrio alginolyticus* harboring the *trh* gene of *V. parahaemolyticus*[25] that was associated with a food poisoning outbreak. Thus, the risk of illness may be underestimated in the present study.

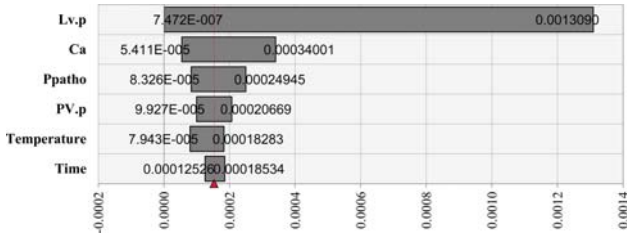

Fig. 2. Effect of input variables on risk associated with consuming raw oyster in Korea, shown as a Tornado plot in the sensitivity analysis. Ca, Consumed amount; Lv.p, level of V.p in raw oyster meat at retail; Ppatho, prevalence of pathogenic gene (*tdh* or *trh*); PV.p: prevalence of V.p in raw oyster; temperature, transport temperature; time, transport time.

For risk management options, the quantification of *V. parahaemolyticus* in seafood with seawater/ambient temperature monitoring needs to be conducted to decide oyster harvest season. Controlling transport/storage temperature and time is a realistic management strategy for both the raw oyster industry and consumers to prevent growth of *Vibrio* spp. It was previously reported that *V. parahaemolyticus* can proliferate in harvested raw oysters by 2.9 log CFU/g in 24 h at 26°C in the U.S.[26]. On the other hand, *V. parahaemolyticus* does not grow at temperatures under 14°C[27]. Therefore, the risk of illness caused by *V. parahaemolyticus* is low between November and March since the atmospheric and seawater temperatures are < 15°C in Korea during this period. There are several days in April and October when the average ambient temperature in Korea is > 20°C; therefore, in warmer months, and the shucking process needs to be conducted under 15°C. The oyster samples at the harvest site without shucking process[12,13], showed lower microbial levels than shucked oysters at the same month.

In addition, the uncertainty of predictions based on age and immune state must be addressed. It was previously shown that the rate of raw oyster consumption was higher among older individuals in Korea[8], implying that elderly people who have weaker immune systems have a higher risk of illness, which must be taken into consideration not to underestimate the risk. The public should be educated on sources of and risk associated with *V. parahaemolyticus* to ensure that shellfish are properly handled, including limiting their exposure to ambient temperatures prior to consumption

and thorough heating during cooking[28], especially for the weak immune people.

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국문요약

본 연구에서 소비-섭취 시나리오와 온도-시간의 장염비브리오 생육모델을 활용하여 국내 생굴의 병원성 장염 비브리오균의 위해평가를 실시하였다. 장염 비브리오균의 오염 수준 및 병원성 인자 데이터를 활용하였으며, 국민건강영양조사와 농촌진흥청의 표준레시피를 활용하여 섭취량을 조사하였고 용량반응관계는 Beta-Poisson모델을 활용하였다. 국내 소비자가 생굴을 섭취할 때 병원성 장염 비브리오균으로 발생하는 위해는 식중독이 주로 발생하는 4월, 10월, 11월에 5.71×10^{-5} (5퍼센타일 2.71×10^{-8}, 95퍼센타일 1.03×10^{-4})로 추정되었다. 본 연구에서 생굴의 장염비브리오 위해의 영향인자는 소비시점 생굴의 장염비브리오균의 오염수준, 생굴 섭취량, 병원성 인자(*tdh* or *trh* 유전자)의 존재 여부, 상온의 노출온도 및 시간으로 나타났으며 위해관리방안을 제시하였다.

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