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Original papers

Quantitative microbial risk assessment of *Cryptosporidium* in bivalve samples from Manila Bay, Philippines

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ABSTRACT. In the Philippines, consumption of bivalves is very common due to its year-round availability and cheap price. However, many consume bivalves as lightly-cooked or raw. This might pose health hazards because bivalves are filter-feeders which act as vehicles for transmission of several pathogens such as the protozoan parasite *Cryptosporidium,* the causative agent of cryptosporidiosis. Cryptosporidiosis in humans is manifested by profuse diarrhea and abdominal pain. To determine the risk of acquiring cryptosporidiosis from consumption of bivalves, quantitative microbial risk assessment (QMRA) should be done. This study aimed to determine the risk associated with the consumption of bivalves which are contaminated with *Cryptosporidium* oocysts. The results indicate that consumption of at least 21 grams of cooked bivalves contaminated with at least 0.1% viable oocysts might pose a risk to consumers, especially to immunocompromised individuals. This estimated risk of infection exceeded the United States Environmental Protection Agency (US EPA) standards (1.0×10^{-4}). Results call for drive of decision-makers to establish an educational or treatment program to reduce the incidence of gastrointestinal infections of the consumers. Improvement of sanitation techniques and hygienic practices will contribute to the decrease of occurrence of the disease.

Keywords: bivalves, *Cryptosporidium,* Manila Bay, Protozoa, quantitative microbial risk assessment

Introduction

Cryptosporidium is a gastrointestinal protozoan parasite from Phylum Apicomplexa, which causes the disease cryptosporidiosis. Symptoms of the disease include profuse diarrhea, abdominal discomfort, mild fever, and vomiting [1]. Immunocompromised individuals, children, pregnant women and elderly are at high risk for this disease, manifesting symptoms more severely while immunocompetent ones recover from the disease in a shorter period [2]. Furthermore, cryptosporidiosis can potentially shorten the life span of immunocompromised individuals [3].

The different routes of transmission of the pathogen make it difficult to control the spread of the disease [4]. Waterborne transmission from fecal matters contaminated by the pathogen is one of the most common routes of infection. Furthermore, *Cryptosporidium* produces oocysts, which can withstand harsh environments and can persist in surface waters for extended periods of time [5]. Once the oocysts are ingested by a potential host, successful infection leading to cryptosporiodiosis may occur.

Bivalves are filter-feeders which can act as bioindicators of the environment for fecal-contaminated waters. Furthermore, bivalves can act as vehicles for infectious diseases because several pathogens can adhere to its tissues [6,7]. A study also reported that bivalves can be responsible for the outbreaks of gastrointestinal diseases [8]. In the Philippines, bivalve consumption is very common due to its high supply, year-round availability, and relatively cheap price. Many Filipinos even prefer eating them lightly-cooked or raw, which is very alarming because *Cryptosporidium* oocysts may still survive even after steam cooking or light fire [7].

Recent studies have reported the occurrence of *Cryptosporidium* in bivalve samples collected from Manila Bay, Philippines [9,10]. These studies can be used to assess the risk of infection of *Cryptosporidium* due to bivalve consumption. Risk assessment was initially developed in the 1970s by the National Academy of Sciences/National Research Council to address chemical hazards which impact human health [11–14]. Quantitative microbial risk assessment (QMRA) identifies the hazards, assesses the exposure and dose-response, and characterizes the risk associated with the ingestion of specific microbe which can cause disease. It is a significant tool in management strategies in connection to water quality and public health [13,15]. Due to the limited studies of *Cryptosporidium* and cryptosporidiosis in the country, and because QMRA is a relatively new technique, it is not yet well-utilized in the Philippines. To the best of the authors' knowledge, this is the first study in the Philippines to conduct QMRA of *Cryptosporidium* in bivalves.

To fill in the research gap, this study aimed to estimate the annual risks of infection of *Cryptosporidium* to people who regularly consume bivalves. Further, two different scenarios were considered, both the consumption of well-cooked and lightly-cooked bivalves.

Materials and Methods

Sampling sites and sample collection. Sampling sites and samples collected for this study were based on the previous work of Pagoso and Rivera [9]. In brief, bivalves were collected from

Table 1. Parameters, assumptions and references used in this study to estimate the daily and annual risks of infection for consuming *Cryptosporidium*-contaminated bivalves

	Type of bivalve consumed	
Parameter	Raw bivalves (untreated)	Cooked bivalves (subjected to: 70°C, 60 min) [18]
	$N = C \times R^{-1} \times I \times V$	$N = C \times R^{-1} \times I \times V \times 10^{-LR}$
	Daily ingestion of *Cryptosporidium* oocysts	
	Average concentration of *Cryptosporidium*: 15.12 oocysts per gram of bivalves [9,19]. Becomes **variable** depending on consumption of contaminated bivalves (100% =15.12, 50% =7.56, 10% =1.512, 1% =0.1512)	
R	**Recovery coefficient: 0.81** [5]	
	Variable: assuming viable oocysts occur at (0.1, 1.0, 10, 20, 40, 60, 100%)	
	Average consumption of bivalves: 5.77 gram per day [20]	
LR	No log reduction (untreated)	Estimated as **1 log reduction (10^{-1})**; – only 11.2% viable oocysts remain after treating of 70°C, 60 min [18]
Risks	$\boldsymbol{P_d = 1 - \exp(-N/K)}$ $\boldsymbol{P_t = 1 - (1 - P_d)^t}$	
	Sample computations:	
	<u>Worst-case scenario (WCS)</u>: Assuming a person consumed **100%** of the average daily consumption, all raw, all viable: $\boldsymbol{N} = 15.12 \times (0.81)^{(-1)} \times 0.41 \times 5.77 = \mathbf{107.71}$	
	<u>Best-case scenario (BCS)</u>: Assuming a person consumed **1%** of the average daily consumption, all cooked, only 1% of the oocysts are viable:	
	$\boldsymbol{N} = 15.12 \times (0.81)^{(-1)} \times 0.001 \times 0.1 \times 5.77 = \mathbf{1.08 \times 10^{-4}}$	
P_d	(<u>WCS</u>): $P_d = 1 - \exp(-107.71/35.7) = \mathbf{0.95}$ (<u>BCS</u>): $P_d = 1 - \exp(-0.107/35.7) = \mathbf{0.003}$	
P_t	(<u>WCS</u>): $P_t = 1 - (1 - 0.95)^{365} = \mathbf{1}$ (certain infection) (<u>BCS</u>): $P_t = 1 - (1 - 0.003)^{365} = \mathbf{0.668}$	

different fish ports around Manila Bay, Philippines, such as Obando, Navotas, Malabon, Parañaque, Las Piñas, and Cavite. *Cryptosporidium* oocysts were concentrated via sucrose flotation [9] and were isolated using Immunomagnetic Separation (IMS).

Exposure assessment. Exposure assessment was performed to estimate the average exposure (N) using Equation 1 [16]: $\boldsymbol{N = C \times R^{-1} \times I \times V}$ where C is the concentration of *Cryptosporidium* oocysts per gram of bivalves, R is the recovery efficiency of the method, I is the fraction of detected pathogens capable of mounting a successful infection, and V is the daily consumption of bivalves.

Quantitative microbial risk assessment. The exponential dose-response (Equation 2) model was used to determine the probability of infection from ingesting various numbers of *Cryptosporidium*-contaminated mussels [17]: $\boldsymbol{P_d = 1 - exp\ (-N/K)}$ where P_d is the probability of infection resulting from daily ingestion of the number of pathogens, N. K is the average number of organisms which must be ingested to initiate an infection. The best-fit K value for *Cryptosporidium* for unknown strains is 35.7 (80% confidence limits) [15].

Estimates of these daily risks may be extrapolated to the risk of infection over extended periods of time (e.g.: 1 year = 365 days) using Equation 3 [17]: $\boldsymbol{P_t = 1 - (1 - P_d)}$ where P_t and P_d are the probabilities of infection after t (365) days and one day of exposure, respectively. The parameters and assumptions for the calculations are listed in Table 1. All calculations were done using MS Excel 2010.

Results and Discussion

The yearly risk estimates of infection from exposure to *Cryptosporidium* via mussel consumption using actual data [9] were shown in Table 2. Two scenarios were accounted, the consumption of raw or lightly-cooked and well-cooked bivalves. All these risk estimates assume an exposure of 365 days within a year (*i.e.:* daily consumption). Risks of infection ranged from 1.10×10^{-3} when assuming 0.1% infective oocysts from cooked bivalves were ingested to as high as 1 (certain infection) if all oocysts ingested from eating raw bivalves can mount an infection (worst-case scenario).

The infection risks estimated in Tables 2 and 3 may be over- or underestimates. Risks may be overestimated by assuming all the *Cryptosporidium* species detected were able to infect humans and by assuming the daily consumption of bivalves. It is unlikely that all *Cryptosporidium* oocysts detected in the bivalve samples are human pathogenic species. So far, two species of *Cryptosporidium*, *C. parvum* and *C. hominis* are responsible for most human infections [5]. The method employed in previous study used the filtration/IMS/FA described in US EPA Method 1623 which does not differentiate among the species of the said organism [9].

Determining acceptable levels of risk would allow for the identification of appropriate pathogen threshold levels on the mussels and other shellfish to be sold at wet markets. Wet markets are open spaces where most commodities are perishable food products, like fresh vegetables, meat, and seafood [21]. Most Filipinos shop at wet markets because these offer cheaper prices [22]. The US EPA

Table 2. Annual risks of infection in different exposure scenarios involving bivalve consumption

Annual consumption (gram) of contaminated bivalves	21.06 (1%)		210.61 (10%)		1, 053.025 (50%)		2, 106.05 (100%)	
Viable oocysts ingested (%)	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw
0.1	1.10×10^{-3}	1.10×10^{-2}	1.10×10^{-2}	1.04×10^{-1}	5.36×10^{-2}	4.23×10^{-1}	1.04×10^{-1}	6.68×10^{-1}
1	1.10×10^{-2}	1.04×10^{-1}	1.04×10^{-1}	6.68×10^{-1}	4.23×10^{-1}	9.96×10^{-1}	6.68×10^{-1}	1
10	1.04×10^{-1}	6.68×10^{-1}	6.68×10^{-1}	1	9.96×10^{-1}	1	1	1
20	1.98×10^{-2}	8.89×10^{-1}	8.89×10^{-1}	1	1	1	1	1
41	3.63×10^{-1}	9.89×10^{-1}	9.89×10^{-1}	1	1	1	1	1
60	4.84×10^{-1}	9.99×10^{-1}	9.99×10^{-1}	1	1	1	1	1
100	6.68×10^{-1}	1	1	1	1	1	1	1

Table 3. Annual risks of infection in assuming a very low occurrence of *Cryptosporidium* (one oocyst per gram of bivalve)

Annual consumption (gram) of contaminated bivalves	21.06 (1%)		210.61 (10%)		1, 053.025 (50%)		2, 106.05 (100%)	
Viable oocysts ingested (%)	Cooked	Raw	Cooked	Raw	Cooked	Raw	Cooked	Raw
0.1	7.28×10^{-5}	7.28×10^{-4}	1.23×10^{-5}	7.26×10^{-3}	3.64×10^{-5}	3.58×10^{-3}	7.26×10^{-3}	7.02×10^{-2}
1	7.28×10^{-4}	7.26×10^{-3}	1.23×10^{-4}	7.02×10^{-2}	3.64×10^{-4}	3.05×10^{-1}	7.02×10^{-2}	5.17×10^{-1}
10	7.26×10^{-3}	7.02×10^{-2}	1.23×10^{-3}	5.17×10^{-1}	3.63×10^{-3}	9.74×10^{-1}	5.17×10^{-1}	9.99×10^{-1}
20	1.45×10^{-2}	1.36×10^{-1}	2.46×10^{-3}	7.67×10^{-1}	7.26×10^{-3}	9.99×10^{-1}	7.67×10^{-1}	1
41	2.94×10^{-2}	2.58×10^{-1}	5.04×10^{-3}	9.50×10^{-1}	1.48×10^{-2}	1	9.50×10^{-1}	1
60	4.28×10^{-2}	3.54×10^{-1}	7.37×10^{-3}	9.87×10^{-1}	2.16×10^{-2}	1	9.87×10^{-1}	1
100	7.02×10^{-2}	5.17×10^{-1}	1.23×10^{-2}	1	3.58×10^{-2}	1	9.99×10^{-1}	1

recommended that microbial risks of infection should not exceed 1×10^{-4} per year [23]. This is equivalent to <1 infection per 10, 000 individuals. Calculated human health risks based on exposure levels to *Cryptosporidium*-contaminated mussels can be compared to the US EPA's recommendations to ensure that the risks are of insignificant levels.

Based on the results of the study, annual consumption of at least 21 grams of cooked bivalves contaminated with 0.1% viable oocysts exceeded the US EPA standards. This result is very alarming because it may indicate both poor water quality in Manila Bay and poor bivalve quality from the propagation sites. On the other hand, this study further confirmed that cooking of bivalves greatly reduces the risk of infection.

Contamination of bivalves in their propagation sites may be caused by anthropogenic activities due to increased human activities and settlements. Some of the contaminants include wastewater, solid wastes, and runoffs which can eventually end up in Manila Bay. A study reported that residents who do not have proper sewage systems discharge their wastes directly to Manila Bay [24]. Also, raising animals such as cows near the area of bivalve propagation sites may also contribute to water pollution. Several studies reported that increased pollution due to farm wastes and calving activities corresponds to seasonal increase of incidence of cryptosporidiosis [25,26]. In low-income settings where surface and ground water are usually used for personal and domestic purposes, contamination with fecal pathogens poses a great risk to public health [27].

Aside from anthropogenic activities, another factor which may affect the occurrence of cryptosporidiosis is the precipitation and temperature in moist tropical countries. The Philippines is a tropical country which serves as an optimal environment for the parasitic protozoan to propagate [28].

The results of this study also reflect similar ranges of risks associated with consumption of various fresh produce (tomatoes, bell peppers or paprika, cucumbers, and lettuce). Presence of 200 oocysts/L of irrigation water can cause 4.48×10^{-3} yearly risk of infection associated with consumption of lettuce [29].

In this study, another scenario was assumed, where a very low occurrence of oocysts (one oocyst per gram of bivalve) was used. The annual risks have decreased and some of the values have met US EPA standards. For example, the best-case scenario, where a person consumes 1% of the average annual consumption which contains 0.1% viable oocysts, the annual risk is 7.28×10^{-5}.

In conclusion, the estimated risks of infection as shown in Table 2 exceeded the US EPA's guideline of 1×10^{-4} per year. This may indicate poor water quality in the mussel cultivation sites. Such high values of infection risk estimates may lead for a cultivator to re-evaluate the sanitation of the water environment and treatment options. However, infection risks may be over- and underestimated depending on the actual yearly consumption value of bivalves per individual. Lastly, the public is urged to prepare well-cooked mussels and other shellfish for consumption because cooking of bivalves allow 1 log reduction of annual risks from consumption of raw bivalves. Therefore, cooking bivalves before consumption is very important in minimizing the risks of infection. Reduction of diarrhea and other gastrointestinal diseases can be done by improving rural sanitation and practices [27]. Thus, the results of this study can be used to improve the sanitation guidelines in Manila Bay and in mussel propagation sites in the area. This can lead to better food quality and lesser occurrence of cryptosporidiosis and other food- and water-borne gastrointestinal diseases. It is recommended that more studies be done to conduct risk analysis programs in other similar communities in the Philippines.

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