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Risk Assessment of *Listeria monocytogenes* in Queso Fresco in Culiacan, Mexico

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Abstract *Listeria monocytogenes* in queso fresco (QF) is a major concern due to its popularity. How to translate microbial contamination data for impacts on public health such as illness probabilities and cumulative risks is in great need in developing countries for decision-making in regulating food safety. The aims of the study were to evaluate the prevalence and types of Listeria in QF and to demonstrate thatquantitative microbial risk assessment (QMRA) can be applied to estimate the cumulativelisteriosis risks from QF consumptionin the Northwestern State of Sinaloa, Mexico. The results illustrate the pressing needs to improve sanitation practices during traditional production of QF to decreaselisteriosis on public health.

Keywords Quantitative Microbial Risk Assessment, Listeria Monocytogenes, Queso Fresco

1. Introduction

Queso Fresco (QF) is an essential ingredient in Mexican cuisine characterized by its snow white color, crumbly texture, lightly salty flavour[21], and consumed without the holding requirement for a raw milk cheese sold in the United States[5]. The production of QF in Latin-America countries is commonly achieved by the enzymatic coagulation of raw milk with rennet and coagulation enzyme and sold in small (down the trade) markets, and/or door-to-door where the cheese may or may not be stored at adequate refrigeration temperature. The safety of QF made with raw milk is still in debate. While it can be made and sold in Latin American countries, is also being considered a safety risk. In the United States, some outbreaks associated with QF contaminated with *L. monocytogenes* have been well documented due to the increasing Hispanic population. Latin-style soft cheese from Central and South America has been also associated with *L. monocytogenes*[1,6,17]. A commonly used method for detecting *L. monocytogenes* from food sources in Mexico is the "Mexican official regulation" including NOM-143-SSA1-1995[19], which is implemented by the big national food processors. However, small producers do not enforce it as good manufacturing practices. Furthermore, the uses of traditional methods by small processors are contributing factors favoring contamination and survival of *L. monocytogenes* and other pathogens in QF[1, 18]. The relatively high moisture content (55 to 58%), low pH levels (pH 5 to 6.3), and low salt content (1.4-1.6%)[21] as well as hand manipulation during processing, distribution and selling increase the likelihood of *L. monocytogenes* contamination. Although pasteurization of milk greatly reduces the risk of *L. monocytogenes*, it is often omitted during non-commercial, unregulated production of fresh, non-aged cheese[16]. Cheese made from pasteurized milk results in a more uniform product of better sanitary quality; however pasteurization adversely affects its flavor quality, since it eliminates some of the indigenous microflora in the milk, which is partly responsible for the development of the typical cheese flavor. It is the reason that some producers and consumers of QF usually prefer the organoleptic qualities of cheeses using raw milk over pasteurized milk. Information related with the number of human listeriosis cases associated with the consumption of QF in Latin American countries such as Mexico is unknown. Quantitative Microbial Risk Assessment (QMRA) can be

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used to predict the probability of *L. monocytogenes* infection and illness in QF consumers. The QMRA is a four-tiered approach that includes hazard identification, exposure assessment, dose-response assessment, and risk characterization[11, 2] and interprets environmental monitoring data for public health impact. For this reason, the aims of this study were to evaluate the prevalence and types of *Listeria* that may be present in QF obtained from markets in the Northwestern State of Sinaloa, Mexico, and to demonstrate that a simple quantitative microbial risk assessment (QMRA) can be applied to estimate the cumulative listeriosis risks from the QF consumption in the Northwestern State of Sinaloa, Mexico with a population of 793,730.

2. Materials and Methods

2.1. Sample Collection

Individual QF samples were obtained from 75 independent merchants within Culiacan city limits. The samples were analyzed in the Food and Environmental Microbiology Laboratory in the Centro de Investigación en Alimentación y Desarrollo (CIAD), Culiacan, Sinaloa, Mexico within 24 h of collection. *L. monocytogenes* was recovered using the FDA-Bacteriological Analytical Manual Standard enrichment/recovery method[8] and positive *Listeria* isolates were sent to the Department of Agriculture-Agricultural Research Service-Eastern Regional Research Center (USDA–ARS-ERRC) for the biochemical and molecular characterization of *L. monocytogenes*.

2.2. Exposure Assessment

Exposure assessment addresses the likelihood that an exposure to a hazard will occur; it also addresses the quantity of the hazardous substance in the exposure. Although the exact route of contamination of QF with *L. monocytogenes* is not known, several sources have been proposed, including the use of contaminated raw milk, contaminated processing surfaces, water and air, and contamination by workers[17]. Three main factors were assumed in this assessment: (i) the amount of *L. monocytogenes* in the exposure; (ii) the amount of cheese consumed; and (iii) the consumers' exposure frequency. The exposure assessment of this study assumes a scenario to estimate the risk from consuming QF made from raw milk. Because *L. monocytogenes* was recovered by a presence and absence method, the scenario of this assessment assumes the same concentrations of *L. monocytogenes* as those in the study of Bemrah et al.[2] to address the exposure to *L. monocytogenes* in QF (Table 1). They calculated a lower and higher concentration of $<10^2$and 10^5 CFU/g of *L. monocytogenes* in soft cheeses by enrichment and modification of the U.S. Food and Drug Administration methodology. For this QMRA we have improved upon the method of Bemrah et al.[2] by considering also the lower, upper, and mean concentration 10^2, 10^3, 10^5 CFU/g of QF (Table 1). Bemrah, et al.[2] also showed the positive rates of *L. monocytogenes* in cheeses to range from 0.5 to 10%, for a typical cheese serving of 31 g. The estimated probabilities of consuming a dose of *L. monocytogenes* greater than 10^2,10^3 and 10^5 were 41%,8.3% and 0.08%, respectively. The amount of *L. monocytogenes* in QF servings was assumed to be random, and the dose was dependent on the amount of *L. monocytogenes* present per gram of QF.

Table 1. Defined Parameters for Exposure of *Listeria monocytogenes* in Queso Fresco

Parameter	Assumption
Listeria monocytogenes/ g of Queso Fresco	$<10^2$, 10^3, 10^5 CFU/g [ab]
Amount of Queso (g) consumed per serving in Latin America	4.5g / day[c]
Annual serving per healthy and immunocompromised population	21.9
Annual serving per elderly population	16.7
Immunocompromised individuals	20%[d]
Virulent strains of *L monocytogenes*	0.1[e]

[a] Bemrah *et al.*[2]
[b] Colony forming units
[c] Lake *et al.*[14]
[d] Gerba *et al.*[10]
[e] Farber *et al.*[7]

The risk assessment estimates for the total number of annual servings of QF consumed by healthy, immunocompromised and elderly population in Culiacan are presented in Table 1. To determine the amount of QF that an individual consumes daily, the GEMS/Food regional diet (regional per capita consumption of raw and semi-processed agricultural commodities) was reviewed for this assessment[22]. The data indicated that the typical serving for the Latin American population is approximated 4.5 g/day (Table 1). Bemrah et al.[2] found that accurate data on individual consumptions patterns of raw milk soft cheese were not available. Due to the lack of knowledge about the number and size of QF servings in Culiacan, Sinaloa, Mexico, this assessment was determined using the data from the National Nutrition Survey (NNS) which uses the data from a study conducted in the Netherlands, where 279 individual dietary records were deemed to represent consumption of a serving of soft cheese from a total survey population of 4,636 on a daily basis[14].

According to the most recent Mexican population Census in 2005[13], Culiacan has a population of 793,730. Among them, 634,984 were identified as healthy individuals and 13,359 individuals were over 65 years old (elderly)[13]. According to Gerba et al.[10] 20% of the USA population represents the high-risk population. For this exposure assessment this percentage was applied to the Culiacan population, and 145,387 individuals were considered immunocompromised.

2.3. Number of Servings of QF and Serving Size

2.3.1. Healthy and Immunocompromised Population

Annual number of servings (healthy and immunocompromised population)

$=279 \times 780{,}371/4{,}636 \times 365 = 1.7 \times 10^7$ servings (1)

Based on an overall population of 780,371, the number of servings per person per year is 21.9, which is similar to the results obtained from the USA and New Zealand, in which the number of servings per person per year was 21.7, and 21.9, respectively[14].

2.3.2. Elderly Population

In this assessment the elderly population will be considered as a separate group from the healthy and immunocompromised individuals. This group is considered to have increased susceptibility to listeriosis due to the physiological changes associated with the natural aging process[9]. From the NNS, 50 out of 297 individual dietary records were deemed to represent consumption of a serving of soft cheese for an individual aged 60 years or more. A total of 1,087 people aged 60 years or more completed dietary recall questionnaires as part of the NNS[14]. According to the Mexican Census[13], 13,359 individuals in Culiacan were aged 65 or older.

The annual number of servings (elderly population)

$= 50 \times 13{,}359 / 1{,}087 \times 365 = 2.2 \times 10^5$ servings (2)

The number of servings per person per year in the elderly population is 16.7. Assessment of how the number of consumed servings per capita and per year influenced the incidence of human listeriosis was made using 21.9 serving for healthy/immunocompromised population and 16.7 serving for the elderly.

2.4. Dose Response Assessment

To address a single exposure to *L. monocytogenes* in QF, the generated data were fitted in a dose-response model by using the Weibull-Gamma equation suggested by Farber et al.[7].

$$Pi = 1 - [1 + (N)b / \beta]^{-\alpha} \quad (3)$$

where Pi is the probability of illness for an individual exposed to N dose of *L. monocytogenes* cells ingested, and α, ß and b are parameters associated with dose-response. In establishing this relationship, factors such as the pathogenicity of the strain and the vulnerability of the host are considered. Healthy individuals rarely develop clinical listeriosis symptoms after consuming contaminated food. For all populations, the parameters α = 0.25 and b = 2.14 were used. ß of $10^{15.26}$ and ß of $10^{10.98}$ were used for the healthy population, and for the elderly and immunocompromised population, respectively[2].

The annual risks of infection were estimated using the following formula,

$$CR = 1 - \prod_{i=1}^{C} (1 - Ri) = 1 - \prod_{i=1}^{C} (1 - V \times Pi) \quad (4)$$

where CR is the annual cumulative risk, V is the proportion of virulent *Listeria* strains, Pi is the probability of illness for an individual exposed to a dose (N) of *L. monocytogenes* cells. The probability of illness linked to the consumption of one cheese serving (Ri) was calculated using Pi times the probability that the consumed strains of *L. monocytogenes* are virulent. The probability of virulent *L. monocytogenes* strains was assumed to be V=0.1, the upper limit of the range suggested by Farber et al.[7].

3. Results

Table 2. Risk of *L. monocytogenes* Illness by the Consumption of One Serving of Queso Fresco

Population	10^{2a}	10^{3a}	10^{5a}
Healthy	6.54×10^{-11}	9.03×10^{-9}	1.72×10^{-4}
Immunocompromised/ Elderly	1.25×10^{-6}	1.72×10^{-4}	4.84×10^{-1}

[a] Concentration of *L. monocytogenes* (CFU/g) in queso fresco

Table 3. Yearly Cumulative Risk of *L. monocytogenes* Infection Associated with the Consumption of Queso Fresco

Population	10^{2a}	10^{3a}	10^{5a}
Healthy[b]	1.43×10^{-10}	1.97×10^{-8}	3.77×10^{-4}
Immunocompromised[b]	2.73×10^{-6}	3.76×10^{-4}	1.06
Elderly[c]	2.08×10^{-6}	2.87×10^{-4}	0.80

[a] Concentration of *L. monocytogenes* (CFU/g) in queso fresco
[b] Assuming 21.9 servings/person/year; dose-response model V=0.1
[c] Assuming 16.7 servings/person/year; dose-response model V=0.1

Table 4. Estimated Cases of Listeriosis in Culiacan per Serving/Year

Population	10^{2d}	10^{3d}	10^{5d}
	Number of listeriosis cases/year		
Healthy [a]	4.15×10^{-5}	5.73×10^{-3}	109
Immunocompromised [b]	1.81×10^{-2}	25	7.03×10^{4}
Elderly [c]	1.66×10^{-2}	2.29	6.46×10^{3}

[a] 634,984 inhabitants
[b] 145,387 inhabitants
[c] 13,359 inhabitants
[d] Concentration of *L. monocytogenes* (CFU/g) in queso fresco

L. monocytogenes was found in 7 out of 75 QF samples collected in Culiacan, Sinaloa, Mexico with a prevalence of 9.3% (data no shown). The parameters and assumptions for this exposure assessment are shown in table 1. The simulated concentration of *L. monocytogenes* in QF ranged from 10^2 to 10^5 CFU/g with an average of 10^3 CFU/g. In order to estimate the risk of illness, two assumptions were made (i) a serving size of 4.5 g and either a 1 or 21.9 servings in a given year for a healthy and immunocompromised population (ii) a serving size of 16.7 for persons over 65 years of age. Table 2 shows the estimated probabilities of illness of *L. monocytogenes* in healthy, immunocompromised and elderly populations, respectively. The dose-response model estimated a probability of illness with the consumption of one cheese serving ranged from 6.54×10^{-11} to 1.72×10^{-4} with an average of 9.03×10^{-9} for a healthy population, and from

$1.25x10^{-6}$ to $4.84x10^{-1}$ with an average of $1.72x10^{-4}$ for immunocompromised and elderly population. Assuming a consumed serving/year of 16.7 and 21.9 at the same doses (10^2, 10^3 and 10^5 CFU/g) the cumulative risk range from $1.43x10^{-10}$ to $3.77x10^{-4}$ with an average of $1.97x10^{-8}$ for a healthy population and $2.08x10^{-6}$ to 0.80 with an average of $2.87x10^{-4}$ for the elderly (Table 3). Using 20% of the overall Culiacan population as immunocompromised, the yearly cumulative risk ranged from $2.73x10^{-6}$ to 1.06 with an average of $3.76x10^{-4}$ (Table 3). Assuming that QF was contaminated at the levels determined in this assessment and assuming that all individuals consumed one serving/year, the expected cases of listeriosis in Culiacan ranged from $4.15x10^{-5}$ to 109 with an average of $5.73x10^{-3}$ for the healthy population, $1.81x10^{-2}$ to $7.03x10^{4}$ with an average of 25 for the immunocompromised population, and $1.66x10^{-2}$ to $6.46x10^{3}$ with an average of 2.29 for the elderly (Table 4).

4. Discussions

The present study was conducted in Culiacan Sinaloa, located in the Northwestern part of Mexico, where QF is made, by small-unregulated processors, using raw milk and commonly sold in small markets and/or door to door. The presence of *L. monocytogenes* in QF is neither traced by the consumers nor by the health authorities. However, there is an Official Mexican Standards regulation NOM-121-SSA-1994 [20] for dealing with *L. monocytogenes*. This policy establishes that *Listeria* must be completely absent in 25 g of cheese to be considering a safe product for consumption. The results of this study demonstrated that *L. monocytogenes* is present in QF made by unregulated processors in 9.3%, which may represent a high risk to cause illness in susceptible population. Previous studies have shown different levels of *L. monocytogenes* in cheeses[1, 2, 6, 17]. Moreno-Enriquez et al.[17] found 3.4% of QF from Mexican retail markets positive for *L. monocytogenes*. The FDA-CFSAN[9] found 1.4% of fresh soft cheese samples positive for *L. monocytogenes*; and Bemrah et al.[2] found a prevalence of 0.5-10% of *L. monocytogenes* in cheeses. Even though the presence of *L. monocytogenes* in cheeses does not necessarily result in developing of illness in healthy population, it may represent a high risk for the susceptible population, such pregnant women, immunocompromised and elderly. According to the CDC[3] the national health target for *Listeria* infection is 0.24 per 100,000 healthy populations and 0.82 per 100,000 for the elderly, which coincide with the actual risk occurrence of the present study.

Two risk assessments of *L. monocytogenes* in soft cheese were identified and compared with this QMRA. The FDA-CFSAN[9] predicted the risk of *L. monocytogenes* for the US population in 23 different ready to eat food, including fresh soft cheeses; and Bemrah et al.[2] risk assessment modelled the risk of *L. monocytogenes* infection from consumption of raw milk soft cheeses in France. Neither of the two studies referred to the risk in Latin American countries. In addition, assumptions and methods employed were different and neither of them addressed the use of raw milk cheese made by artisanal techniques. The estimated risk for this assessment was slightly higher than the risk obtained by Bemrah et al.[2] in which the probability of illness associated with one soft cheese serving was predicted to range from zero to $1.96x10^{-8}$ for healthy population and from zero to $3.73x10^{-4}$ for a high risk subpopulation. The FDA-CFSAN[9] estimated annual listeriosis cases were less than 0.1 for fresh soft cheeses. The risk estimated was lower compared to our results. However, that risk was based on chesses purchased at retail stores made with pasteurized milk and not with raw milk as assessed in the present study. On the other hand, the estimated probability of fresh soft cheeses contaminated with *L. monocytogenes* might increase 50% when soft cheeses are made using unpasteurized milk, which is in accordance with our results[9]. The number of human listeriosis by Bemrah et al.[2] ranged from 34 to 90 with 50 million inhabitants and a serving size of 50/capita/year. If our study is extrapolated to Bemrah et al.[2] the human listeriosis cases in Culiacan region are similar to their results.

Regarding to assumption, the infection risk of this assessment may be over- or-under estimated. Risk may be overestimated by assuming that the entire population of Culiacan consumed QF, that all QF samples were contaminated a concentration of 10^2, 10^3 and 10^5 CFU/g of *L. monocytogenes*, and that infection with *L. monocytogenes* is associated with only a few virulent strains (factor 0.1) as suggested by Bemrah et al.[2] and Farber et al.[7]. Also, this QMRA assumes the contamination during cheese preparation is similar when cheese is made from pasteurized milk, temperature did not affect produce quality and that *L. monocytogenes* growth is constant. Risk might be underestimated by assuming that each consumer eats 4.5 g of QF per day given the popularity of this product in Latin American countries, and that 20% of Culiacan population is immunocompromised. The probability that more than 20% of Culiacan population falls into the high-risk category enables us to determine that the infection risk of this assessment is conservative. Furthermore, influence of QF quality for cross-contamination from transporting between retailer and consumer and the time between production and consumption, were not considered in this QMRA.

Despite the inherent limitation of this QMRA process, the present study is the first effort to estimate the risk infection for consumption of QF contaminated with *L. monocytogenes* in Culiacan, Mexico. It shows the need for standardized protocols among independent merchants to enhance the quality and safety of the QF product. Greater insight on prevalence, levels, and types of *Listeria* in soft cheese will augment efforts to better manage the threat of listeriosis, especially since the data of cases of listeriosis in Culiacan, Sinaloa, are unknown.

5. Conclusions

QF is considered a risky food that has been associated with the presence of different pathogenic microorganisms such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, and *campylobacter*, however, the greatest concern has been focused on *L. monocytogenes*. The control of *L. monocytogenes* in QF made with raw milk is difficult; however, improved sanitation practices during traditional production should minimize the likelihood of product contamination. The present study is the first attempt to estimate risk of infection of QF contaminated with *L. monocytogenes* in a Mexican population in which the demand and consumption of QF is high. Because foodborne outbreaks associated with QF are unknown in Mexico, this model can be used to implement risk of listeriosis infection in different population (healthy, immunocompromised and the elderly) of similar regions. The fact that QF production will continue to be permitted in developing countries, such as Mexico, better efforts for food safety regulation and communication of risk to the health authorities are needed to improve the hygiene during the artisan production of QF. Finally, maintaining and monitoring consumer's safety are of primary concern.

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