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Orginal Article

Vibrio parahaemolyticus in fish and shellfish of animal origin from establishments in the port of Chicxulub, Yucatan, Mexico

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Abstract

Introduction: *Vibrio parahaemolyticus* is a human pathogen that is widely distributed in marine environments. This organism is frequently isolated from a variety of raw seafood products, particularly fish and shellfish. Consumption of raw or undercooked fish and shellfish contaminated with *Vibrio parahaemolyticus* can lead to the development of acute gastroenteritis characterized by diarrhea, headache, vomiting, nausea, and abdominal cramps. It has also been isolated from wound infections and septicemias. Most cases are non–fatal. This bacterium is recognized as the leading cause of human gastroenteritis associated with fish and shellfish consumption in the United States of America and an important fish and shellfish borne pathogen worldwide. Chile faced its first outbreak in 1997–1998. Subsequently, outbreaks and cases have continued to occur, all associated with the consumption of fish and shellfish. **Objective:** To determine if raw seafood, marinated without heat, partially cooked with heat and completely cooked with heat that are sold for human consumption in establishments in the port of Chicxulub, Yucatan, Mexico, represent potential risk factors for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia by *Vibrio parahaemolyticus* species. **Material and methods:** Study conducted on a representative sample selected from the total of two hundred samples from thirty–eight establishments. From July 1 to December 31, 2021, one hundred thirty two samples of seafood were studied. Using the Cornfield Method, the estimation interval was constructed at the 95% confidence level. **Results:** In thirty–seven (28.03%) samples an equal number of strains were isolated whose biochemical characteristics corresponded to *Vibrio parahaemolyticus*. The prevalences obtained in raw marine foods, marinated without heat, partially cooked with heat and completely cooked with heat were 35.59%, 45.45%, 22.45% and 0.00%. The Cornfield estimation interval at the 95% confidence level for *Vibrio parahaemolyticus* was $13.56\% \leq P \leq 42.50\%$. **Conclusion:** Raw seafood, marinated without heat and partially cooked with heat represent potential risk factors for *Vibrio parahaemolyticus* for the development of acute gastroenteritis, wound infection, primary septicemia, and secondary septicemia.

Introduction

The genus *Vibrio* belonging to the family *Vibrionaceae* has sixty–six species of which at least twelve are recognized as human pathogens [1].

Of these, *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* are the most important pathogenic vibrios both in terms of disease–causing capacity and overall disease burden [2].

Vibrio parahaemolyticus is a gram–negative, slightly curved, facultative aerobic, halophilic, oxidase–positive, glucose–fermenting, but not sucrose and variable urease–fermenting bacillus. It requires selective media for its development with a sodium chloride (NaCl) concentration of 3% [1].

In the ninth edition of the Bergey Manual of Determinative Bacteriology the *Vibrionaceae* family is made up of the genera *Aeromonas*, *Enhydrobacter*, *Photobacterium*, *Plesiomonas* and *Vibrio*. Of the sixty–six accepted *Vibrio* species, at least fifteen have been isolated from clinical samples and the following twelve are considered pathogenic: *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*,

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Vibrio furnissii, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio parahaemolyticus* [3].

Vibrio parahaemolyticus causes both food transmission and injury infections throughout the world and in the United States of America has the highest mortality rate of all food pathogens transmitted by food. According to estimates, both the Centers for Disease Control and Prevention (CDC) of the United States of America as of the Food and Drug Administration (FDA), there are fifty cases of food transmission per year in the United States of America serious enough to require hospitalization Although up to forty one thousand cases have been calculated per year [4].

Vibrio parahaemolyticus has also been isolated from seawater and/or has been implicated as a source of infections (mainly wounds) in Denmark, Sweden, Germany, the Netherlands and Belgium [5].

Vibrio parahaemolyticus species is highly invasive and causes fulminant primary septicemia in people at risk of infection with mortality rates of approximately 60% [6].

Infection leading to primary sepsis is associated with the consumption of raw shellfish contaminated with *Vibrio parahaemolyticus*, especially raw oysters, and sepsis symptoms typically develop within twenty four hours of ingestion. In fatal cases, death can occur within hours of admission to the hospital. Individuals who are immunosuppressed or have elevated serum iron levels, typically due to a disease that causes chronic liver damage (such as cirrhosis of the liver or viral hepatitis), are at increased risk of infection by this organism [7].

In addition, infections occur more frequently in men (82% of the cases reviewed) [6], whose average age exceeds fifty years. The most common symptoms in the form of primary sepsis infection include fever (94%), chills (86%), nausea (60%), and hypotension (systolic pressure < 85 mmHg; 43%).

These values are very similar to those reported [7] in a recent study of three hundred thirty–three patients with *Vibrio* infections associated with eating raw oysters in Florida. It also found that 94% of the patients were hospitalized for up to forty–three days (an arithmetic mean > eight days).

An unusual symptom is the development (in 69% of patients) of secondary injuries, typically of the extremities, often requiring surgical debridement and/or resulting in amputation [6] .

In addition to the primary septicemia that follows ingestion, *Vibrio parahaemolyticus* is known to infect the wounds of otherwise healthy people [6,8].

These typically occur through contamination of pre–existing wounds with seawater or through contact with raw fish or shellfish. Symptoms of this type of infection include localized pain, edema, erythema, and ultimately severe necrosis of the surrounding tissue, often resulting in surgical debridement or amputation [6].

Mortality rates after wound infection are approximately 25% [6,8].

In a review of eleven patients infected with *Vibrio parahaemolyticus* during 1994 in Denmark reported that four developed bacteremia, one of whom died, and nine developed skin lesions [5].

Although apparently present in estuarine and coastal waters throughout the world, the ability to isolate *Vibrio parahaemolyticus* and the frequency of infections (both primary septicemia and wounds) exhibit definite correlations with seawater temperature [6,8].

It is difficult to isolate the bacteria when the water temperature is below 10°C [6,9] and most cases, whether due to ingestion or injury, occur between the months of May and October [6,8].

It has been speculated that this seasonal distribution of infections and isolation reflects the entry of *Vibrio parahaemolyticus* into a viable but uncultivable state [10].

Vibrio parahaemolyticus in mollusks causes the highest mortality rate of any foodborne pathogen in the United States of America. Primary septicemia is the clinical syndrome most frequently associated with foodborne *Vibrio parahaemolyticus* infections. Based on surveillance data, from 1988 to 1996, the CDC estimates that approximately fifty foodborne cases occur annually in the United States of America, but only half of these cases are reported; approximately 40% of reported cases are fatal [11].

Almost all infected people reported previous underlying chronic diseases, particularly liver disease [12] .

Vibrios other than *Vibrio parahaemolyticus* are estimated to cause approximately five thousand foodborne infections per year in the United States of America [11] and *Vibrio parahaemolyticus* is generally considered to be the main cause of these infections [7,13]. Gastroenteritis with occasional bloody diarrhea is the most common syndrome associated with *Vibrio parahaemolyticus* infections, but primary septicemia has been reported in people with underlying chronic disease. At least, with respect to gastrointestinal infections, there does not appear to be a difference in the susceptibility of any population at risk compared to healthy individuals. More than 95% of clinical *Vibrio parahaemolyticus* strains produce a thermostable direct hemolysin encoded by the idh1 gene; this gene is relatively rare in food and environmental isolates [14]. Four recent outbreaks of *Vibrio parahaemolyticus* associated with oysters in the United States of America: Washington State 1997 and 1998, Texas 1998 and New York 1998 [15] concern and interest in this pathogen have increased [16-18]. The incidence of *Vibrio parahaemolyticus* diseases in Asia began to increase in 1996 and is attributed to the appearance of a new strain of serotype O3: K6 [19. The outbreaks in Texas and New York were caused by this strain. In the Texas outbreak, the largest ever reported in the United States of America, an unusually high attack rate was reported [18].

One pathogen that can be transmitted by oysters is *Vibrio parahaemolyticus*. Described in 1976 it was called "*Vibrio* lactose positive", later it was called *Beneckea vulnificus* and finally *Vibrio parahaemolyticus*. It belongs to the *Vibrionaceae* family, they are gram–negative, straight and curved bacilli, mobile due to the presence of a polar flagellum, oxidase positive, not sporulated. They are thermolabile and behave like facultative anaerobes. Among the more than thirty species of the genus *Vibrio*, twelve have been reported as pathogens for man, among which *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* stand out. They grow at a temperature of 37°C with a range of 8°C–43°C at a pH of 7.8 with a range of 5–10 and can optimally survive refrigeration [20].

Vibrio parahaemolyticus is found in oysters, clams, and shellfish from coastal waters or river mouths around the world. This microorganism is also present in sediment, plankton and other forms of marine life; it has been isolated from a wide variety of ecosystems such as the coasts of the Gulf of Mexico, the Atlantic Ocean and the Pacific Ocean [21].

Because they are found in warm marine waters, people with open wounds can be exposed to *Vibrio parahaemolyticus* through contact with marine waters, shellfish, and marine wildlife. There is no evidence of person–to–person transmission of *Vibrio parahaemolyticus* and it is not related to fecal contamination. People who have immunocompromised conditions and especially those with chronic liver disease are particularly at risk of contracting a *Vibrio parahaemolyticus* infection when they eat raw or undercooked fish and shellfish, or if they bathe in marine waters with a cut or scratch. About three–quarters of patients with *Vibrio parahaemolyticus* infections are known to have liver disease or are immunosuppressed. On the other hand, healthy people have a lower risk of *Vibrio parahaemolyticus* infection. Most *Vibrio parahaemolyticus* diseases occur during the summer months [22,23].

Those responsible for the increase in the number of *Vibrio parahaemolyticus* in fishery products at any given time are temperature, pH, salinity and the increase in organic matter, among others. *Vibrio parahaemolyticus* is found on the coasts of the Gulf of Mexico, in oysters and in sea water during the rainy season or when the sea water temperature is high (23°C). It has been estimated that from April to October 40% or more of the oysters caught off the coast of the Gulf of Mexico may contain this pathogen through a symbiotic association between the bivalve and the adhering bacteria. Oysters that are caught in places where temperature and salinity favor the growth of *Vibrio parahaemolyticus* have been indicated to be a risk, since they can be the cause of various clinical pictures. The high concentrations of this microorganism in these bivalves caught off the coasts of the Gulf of Mexico are related to the hottest months. The relationship between salinity and the presence of *Vibrio parahaemolyticus* has not been established, suggesting that summer temperatures and salinity ranges normally found on the shores of the Gulf of Mexico play a significant role in the number of bacterial cells present. Elevated levels of *Vibrio parahaemolyticus* have been observed when the temperature oscillates between 17 and 31°C with a salinity between 15 and 25%. It has been suggested that the temperature and salinity ranges in which this microorganism can be found are wider for the temperature of 8 to 31°C and for the salinity of 1 to 34%. *Vibrio parahaemolyticus* has been implicated in human infections during the summer [24].

The objective of the present study was to determine the prevalences of the *Vibrio parahaemolyticus* species in raw marine foods, marine foods marinated without heat, marine foods partially cooked with heat and marine foods completely cooked with heat, that is, to determine if these foods represent potential factors risk by the species *Vibrio parahaemolyticus* for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia.

Hypothesis formulation

Null hypothesis (H_0)

Raw seafood, marinated without heat, partially cooked with heat and completely cooked with heat are not contaminated with the species *Vibrio parahaemolyticus* and are not, consequently, potential risk factors for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia.

Alternative hypothesis, working hypothesis or research hypothesis (H1)

Raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat are contaminated with the species *Vibrio parahaemolyticus*, thus constituting potential risk factors for the development of acute gastroenteritis, wound infection, primary septicemia and septicemia secondary.

Material and methods

Epistemological approach

Quantitative approach, probabilistic approach or positivist approach [25].

Study design

Cross–sectional descriptive observational epizootiological study with no directionality and prospective temporality [26].

Study universe

Representative sample selected from the total of two hundred samples of the Thirty–eight establishments that sell seafood for human consumption in the city of Chicxulub, Yucatan, Mexico. Said representative sample was taken in the period from July 1 to December 31, 2021.

Chicxulub Puerto

(en maya: Chic Xulub (pronunciar la "x" como "sh") 'Lugar del cuerno prendido') es una localidad mexicana del estado de Yucatán, en el litoral del golfo de México, comisaría del municipio de Progreso. Se encuentra a 8 km al oriente del puerto de Progreso de Castro, a 40 km al norte–nororiente de la ciudad de Mérida, Yucatán, y a 20 km al norte de otra localidad homónima, la cabecera del municipio denominado Chicxulub Pueblo.

Por lo anterior suele confundirse el pequeño puerto de Chicxulub con la localidad interior de Chicxulub Pueblo y con el municipio del mismo nombre. Son tres denominaciones homónimas pero distinguibles entre sí.

Se dice que en 1531 fondeó allí sus naves Francisco de Montejo, también conocido por Montejo el Adelantado; sin embargo, no hay referencias confiables que ratifiquen este decir.

Junto con el cercano puerto de Chuburná, Chicxulub Puerto fue declarado vigía de Yucatán en 1663 por el gobierno de Juan Francisco Esquivel y de la Rosa para la defensa de la costa contra la incursión de los piratas.

Toponimia

Stela pointing to the center of the Chicxulub crater where it is conjectured that the meteorite that caused the extinction of the dinosaurs hit.

Chicxulub literally means in Mayan language, *flea of the devil*. It is derived from the voices *ch'ik,* which means flea, and *xulub*, devil or demon.

Crater de Chicxulub

The genus Vibrio, belonging to the family *Vibrionaceae*, has sixty–six species, of which at least twelve are recognized as human pathogens [1].
Of these, *Vibrio cholerae, Vibrio parahaemolyticus* and *Vibrio vulnificus* are the most important pathogenic vibrios in terms of both disease–causing capacity and overall disease burden [2].

Vibrio parahaemolyticus is a gram–negative, slightly curved, facultative aerobic, halophilic, oxidase–positive,

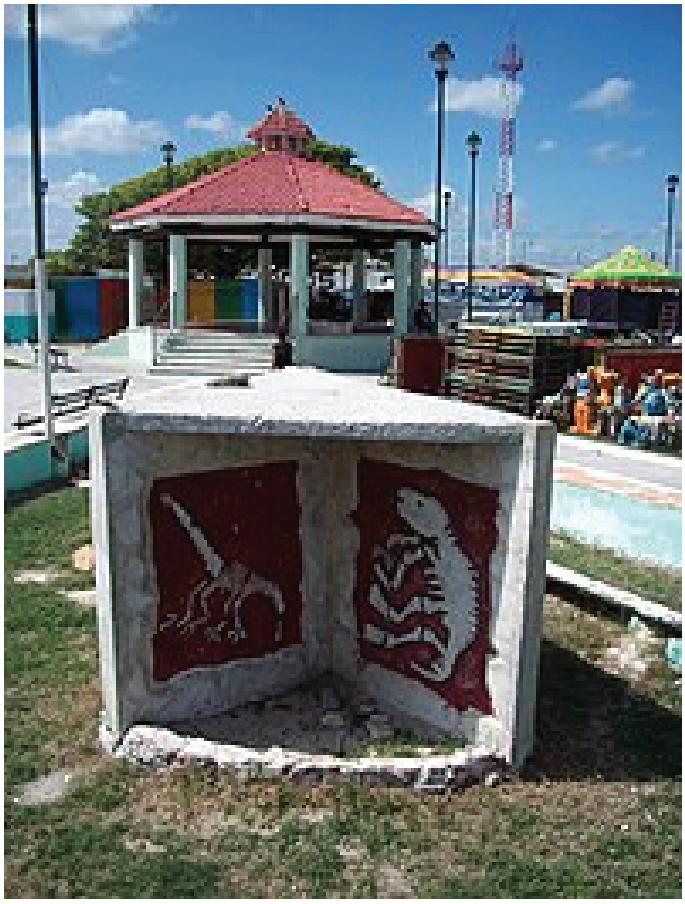

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Figure 1.

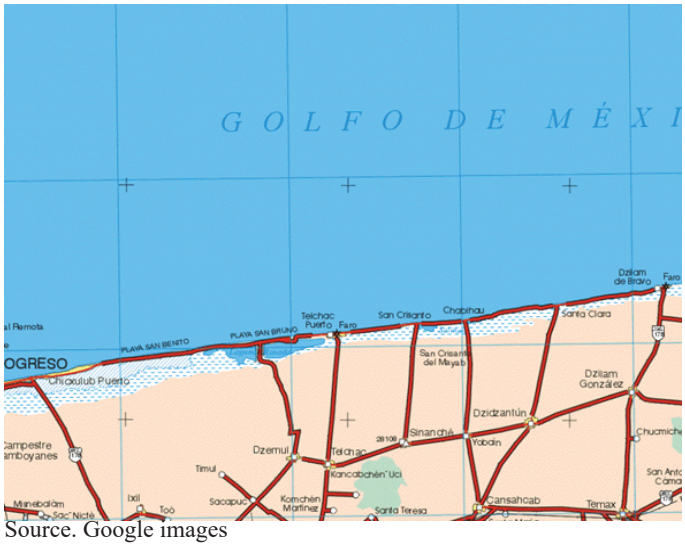

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Figure 2.

glucose–fermenting, but not sucrose–fermenting and variable urease–fermenting bacillus. It requires selective media for its development with a sodium chloride (NaCl) concentration of 3% [1].].

Source. Google images

Figure 1.

Operational definitions of the variables

Establishments: Any establishment that sells marine foods of animal origin for human consumption and that has a health license issued by the Health Services of the state of Yucatan [27].

Marine food: Any product of animal origin from the sea that provides the human body with elements for its nutrition [27].

Raw marine food: Any product of animal origin from the sea that provides the human organism with elements for its nutrition and that at the time of sampling has been found in its natural state [27].

Marinated seafood without heat: Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling have been found cooked using the action of the acid of lemon juice, the acid of orange juice and vinegar, among others [27].

Marine food partially cooked with heat: Any product of animal origin from the sea that provides the human organism with elements for its nutrition and that at the time of sampling has been found prepared in the following way: a) Heat water to boiling; b) Turn off the heat source and add the marine food; c) Let the seafood "soften" in the hot water for five min; and d) Transferring the marine food to a container by letting it rest until cool. This food is ready to be used in the preparation of cocktails and/or ceviches [27].

Completely cooked seafood with heat: Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling has been found cooked using the action of heat (for example: grilled, fried and steam, among others) [27].

Techniques and procedures

A list of thirty–eight establishments that specialize in the sale of marine food for human consumption was obtained. A first visit was made to each of the thirty–eight establishments and compiled a list of two hundred samples. The sampling scheme corresponding to the simple random sampling was used. The sample size was calculated using the following statistician [28]:

$n= NZ^2PQ / d^2(N–1) + Z^2PQ$

Where:

n= sample size; N= population size; Z= level of confidence;

P= proportion of elements in the population that has the characteristic of interest; Q= proportion of elements in the population that does not have the interest characteristic; and d= error level. A level of confidence of 95% was used, that is, a value of z= 1.96; a value of p= 0.5000; a value of q= 0.5000; and a value of d= 0.0500, that is, 5% error level.

$n= 200 (1.96)^2 (0.5000) (0.5000) / (0.0500)^2 (200–1) + (1.96)^2 (0.5000) (0.5000)$

n= 132

Accordingly, one hundred thirty two samples from the list of two hundred of the Thirty–eight establishments were randomly selected. The establishments that corresponded to randomly selected samples received a second visit during which said samples were obtained.

Each sample weighed approximately fifty g; it was stored individually on a sterile polyethylene bag; it was stored in refrigeration and sent to the Departamento de Microbiología. Laboratorios Micro–Clin, S.A. de C.V.

According to the schedule of activities of the research protocol, the processing of the samples was carried out in the period from July 1 to December 31, 2021. For the homogenization and enrichment of each sample, as well as for the isolation and identification of the *Vibrio parahaemolyticus* species, it was according to the methodology described in the eighth edition of the Food and Drug Administration Bacteriological Analytical Manual [29].

Two x two contingency tables were constructed from which the prevalences were calculated. As a test of hypothesis or testing of statistical significance, the Ji–Square Statistic of Mantel and Haenszel ($x^2M–H$) was used. The Epi Info Software for Windows, version 7.1.5.2, was used, for obtaining the values of the statistic's $x^2M–H$ and the probabilities (p). The criterion applied in carrying out hypothetic tests or statistical significance tests for the difference between two proportions was based on the recommendations made by Cochran [30]: a) when $n > 40$ use the $x^2M–H$ test; b) when $20 \leq n \leq 40$ use the $x^2M–H$ test if, and only if, all the expected frequencies are ≥ 5; If at least one cell is at least an expected frequency < 5 use, then, Fisher's Exact Probability Test (PPEF); and c) when $n < 20$ use the PPEF.

$x^2M–H= \Sigma (|O – E| – ½)^2 / E$

$PPEF= (a+b)! (c+d)! (a+c)! (b+ d)! / n! a! b! c! d!$

O= Observed frequencies;

E= Expected frequencies;

(a+b)!= Factorial of (a+b);

(c+d)!= Factorial of (c+d);

(a+c)!= Factorial of (a+c);

(b+ d)!= Factorial of (b+ d);

n!= Factorial of n;

a!= Factorial of a;

b!= Factorial of b;

c!= Factorial of c; &

d!= Factorial of d.

The Cornfield estimation interval was built at the 95% confidence level for the percentage in the seabed population with Vibrio parahaemolyticus. Said estimate interval was built using the following statistician [28]:

$p – Z\sigma p \leq P \leq p + Z\sigma p$

Where:

p= Proportion of elements in the sample that possesses the interest characteristic;

Z= Level of confidence;

σp= Standard error; &

P= Proportion of elements in the population that possesses the characteristic of interest.

At the same time: $\sigma p= pq / n$

Where:

σp= Standard error;

p= Proportion of elements in the sample that has the interest characteristic;

q= Proportion of elements in the sample that does not possess the interest characteristic; &

n= Sample size.

The Cornfield estimation interval at the 95% confidence level for the percentage in the seabed population with *Vibrio parahaemolyticus* was $13.56\% \leq P \leq 42.50\%$.

The eight key differential tests to divide the twelve clinically significant species of the genus *Vibrio* in six groups are presented in Table 1 [31]. The species investigated in the present work belongs to Group 6 (negative production of arginine dehydrolase and positive disarrangement of lysine).

Data processing

In the stage of processing the data were reviewed (information quality control); classified (in qualitative scale); computerized (the Statistical Package for Social Sciences (SPSS) software (SPSS) was used, version 22); presented (in Tables and in Figures); and summarized (the corresponding summary measures were used for classified data in qualitative scale). In the stages of analysis and interpretation, the data was analyzed and interpreted, respectively.

Results

According to its method of preparation, marine foods were ranked, marinated without heat, partially cooked with heat and completely cooked with heat. Three were the varieties (crustaceans, mollusks and fish) and nineteen studied species (shrimp, crab, jaiba, squid, snail, oyster, octopus, abadejo, bulkine, dogfish, crowned, corvine, chihua, mere, pramp, snapper, picuda, blonde and saw).

Table 2 presents the absolute and relative frequencies of marine food by *Vibrio parahaemolyticus* prevalence according to preparation methods.

Figure 1 shows the relative frequencies of marine food by prevalences of Vibrio parahaemolyticus according to preparation methods.

Table 1. *Eight key differential tests to divide the twelve clinically significant Vibrio species into six groups.*

Key differential tests	Group 1		Group 2	Group 3	Group 4	Group 5			Group 6			
	Vibrio cholerae	Vibrio mimicus	Vibrio metschnikovii	Vibrio cincinnatiensis	Vibrio hollisae	Vibrio damsela	Vibrio fluvialis	Vibrio furnissii	Vibrio alginolyticus	Vibrio parahaemolyticus	Vibrio vulnificus	Vibrio carchariae
Growth on nutrient agar with 0% NaCl	+	+										
Growth on nutrient agar with 1% de NaCl	+	+										
Oxidase test			–									
Reduction of nitrates (NO_3) to nitrites (NO_2)			–									
Myo–inositol fermentation				+								
Arginine dehydrolase production					–	+	+	+	–	–	–	–
Lysine decarboxylation					–				+	+	+	+
Decarboxylation of ornithine					–							

Source. [31]

Table 2. *Absolute and relative frequencies of marine food by prevalence Vibrio parahaemolyticus according to preparation methods.*

Preparation methods	Prevalences	Totals
	Vibrio parahaemolyticus	
Raw	21 (35.59%)	59
Marinated without heat	5 (45.45%)	11
Partially cooked with heat	11 (22.45%)	49
Completely cooked with heat	0 (0.00%)	13
Totals	37 (28.03%)	132

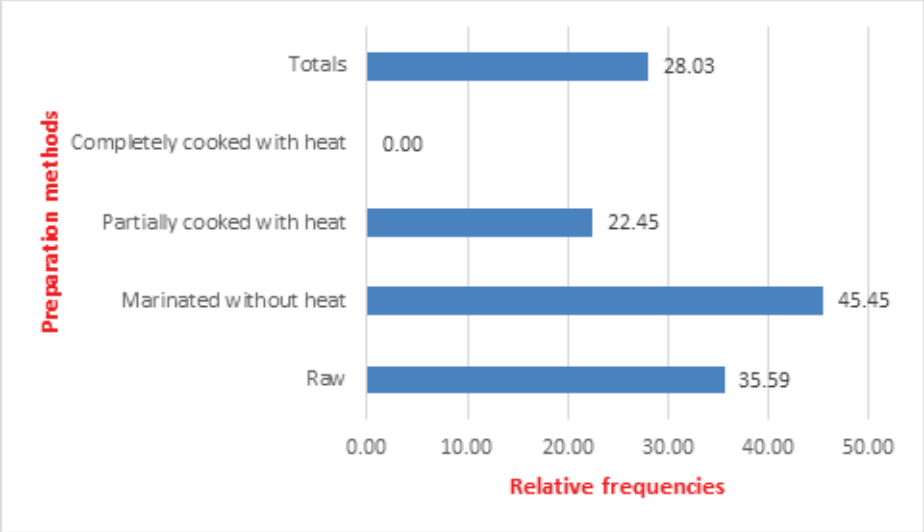
Source. Own elaboration

In thirty–seven (28.03%) samples an equal number of strains whose biochemical characteristics corresponded to Vibrio parahaemolyticus were isolated. The prevalences obtained in raw marine food, marinated without heat, partially cooked with heat and completely cooked with heat were 35.59% (21/59), 45.45% (5/11), 22.45% (11/49) and 0.00% (0/13).

Using the $x^2M–H$ statistic, the corresponding hypothesis contrasts were performed, finding statistically significant differences between the prevalences obtained in raw marine foods versus completely cooked marine foods and between the prevalences obtained in partially cooked marine foods versus marine food completely cooked with heat: $x^2M–H(\alpha= 0.0500, gl= 1) > 3.8416; p < 0.0500$.

Discussion

With respect to the *Vibrio parahaemolyticus* species, the highest prevalence (45.45%) was observed in marinated marine foodless food; therefore, this result corresponds to the expected because they are food that have not been exposed to the action of heat.



Source. Table 2

Figure 4. *Relative frequencies of marine foods by prevalences of Vibrio parahaemolyticus according to preparation methods.*

The next prevalence (35.59%) was observed in raw marine food; consequently, as in heatless marinated marine foods, this result also corresponds to the expected because the probability of isolation is greater when the food has not been exposed to the action of heat.

Below is the prevalence of Vibrio parahaemolyticus (22.45%) observed in marine food partially cooked with heat; this result also corresponds to the expected and the observed prevalence can be explained because the procedure used for "softening" food is not sufficient to destroy the microorganism, or because the food could have been contaminated by the manipulator after the "softening ", either by cross contamination from other food, or by means of the ano–hand–mouth mechanism for being an asymptomatic carrier.

No strain was isolated (0.00%) in the thirteen samples of completely cooked marine foods; subsequently, this result also corresponds to the expected because the probability of isolation is null when the food has been prepared by an adequate exposure to the action of heat.

Conclusion

Based on the observed results, the null hypothesis (H_0) is rejected and alternating hypothesis, work hypothesis or research hypotheses (H_1), i.e., raw marine food, marinated without heat and partially cooked with heat represent factors potentials of risk by the *Vibrio parahaemolyticus* species for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia.

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