# Vibrio vulnificus in seafood of animal origin from establishments in the city of Telchac Puerto, Yucatan, Mexico

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## ABSTRACT

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 city of Telchac Puerto, Yucatan, Mexico, represent potential risk factors for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia by *Vibrio vulnificus* species. Material and methods. Study conducted on a representative sample (n= 132) selected from the total of 200 samples from 38 establishments. From July 1 to December 31, 2019, 132 samples of seafood were studied. Using the Cornfield method, the estimation interval was constructed at the 95% confidence level. Results. In 37 (28.03%) samples an equal number of strains were isolated whose biochemical characteristics corresponded to *Vibrio vulnificus*. 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 vulnificus* was 13.56% ≤ P ≤ 42.50%. Conclusion. Raw seafood, marinated without heat, and partially cooked with heat represent potential risk factors for *Vibrio vulnificus* for the development of acute gastroenteritis, wound infection, primary septicemia, and secondary septicemia.

**Keywords:** *Vibrio vulnificus*, seafood, establishments.

## 1 INTRODUCTION

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 66 accepted *Vibrio* species, at least 15 have been isolated from clinical samples and the following 12 are considered pathogenic: *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Holt JG, Krieg NR, Sneath PHA, Staley JT & Williams ST, "Bergey's Manual of Determinative Bacteriology: subgroup 2: Family *Vibrionaceae*", *Williams & Wilkins*, Baltimore, 1994, pp. 190–194; 259–274).

*Vibrio vulnificus* 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 the Control and Prevention of Diseases of the United States of America as of the Food and Drug Administration, there are 50 cases of food transmission per year in the United States of America serious enough to require hospitalization Although up to 41,000 cases have been calculated per year (Todd ECD, "Preliminary estimates of costs of food–borne disease in the U.S.", *J. Food Protect*, núm 52, 1989, pp. 595–601).

*Vibrio vulnificus* 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 (Dalsgaard A, Frimodt–Møllere N, Bruun B, Høi L & Larsen JL, "Clinical manifestations and molecular epidemiology of *Vibrio vulnificus* infections in Denmark". *Eur. J. Clin. Microbiol. Infect. Dis*. núm 15, 1996, pp. 227–232).

The *Vibrio vulnificus* species is highly invasive and causes fulminant primary septicemia in people at risk of infection with mortality rates of approximately 60% (Oliver JD, "*Vibrio vulnificus. In: Foodborne Bacterial Pathogens (Doyle MP, Ed.)*", 1989, pp. 569–599).

Infection leading to primary sepsis is associated with the consumption of raw shellfish contaminated with *Vibrio vulnificus*, especially raw oysters, and sepsis symptoms typically develop within 24 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 (Hlady WG & Klontz KC, "The epidemiology of *Vibrio* infections in Florida, 1981–1993", *J. Infect. Dis*. núm 173, 1996, pp. 1176–1183).

In addition, infections occur more frequently in men (82% of the cases reviewed) (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed.)", 1989, pp. 569–599), whose average age exceeds 50 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 (Hlady WG & Klontz KC, "The epidemiology of *Vibrio* infections in Florida, 1981–1993". *J. Infect. Dis*, núm 173, 1996, pp.1176–1183) in a recent study of 333 patients with *Vibrio* infections associated with eating raw oysters in Florida. It also found that 94% of the patients were hospitalized for up to 43 days (an arithmetic mean > 8 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 (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens" (Doyle MP, Ed.), 1989, pp. 569–599).

In addition to the primary septicemia that follows ingestion, *Vibrio vulnificus* is known to infect the wounds of otherwise healthy people (Oliver JD, *Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed.), 1989, pp. 569–599; Hlady WG, "*Vibrio* infections associated with raw oyster consumption in Florida, 1981–1994", *J. Food Protect*, núm 60, 1997, pp. 353–357).

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 (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed.)", 1989, pp. 569–599).

Mortality rates after wound infection are approximately 25% (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed.)", 1989, pp. 569–599; Hlady WG, "*Vibrio* infections associated with raw oyster consumption in Florida, 1981–1994", *J. Food Protect*, núm 60, 1997, pp, 353–357).

In a review of eleven patients infected with *Vibrio vulnificus* during 1994 in Denmark reported that four developed bacteremia, one of whom died, and nine developed skin lesions (Dalsgaard A, Frimodt–Møllere N, Bruun B, Høi L & Larsen JL, "Clinical manifestations and molecular epidemiology of *Vibrio vulnificus* infections in Denmark", *Eur. J. Clin. Microbiol. Infect. Dis*. núm 15, 1996, pp. 227–232).

Although apparently present in estuarine and coastal waters throughout the world, the ability to isolate *Vibrio vulnificus* and the frequency of infections (both primary septicemia and wounds) exhibit

definite correlations with seawater temperature (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed)", 1989, pp. 569–599; Hlady WG, "*Vibrio* infections associated with raw oyster consumption in Florida, 1981–1994", *J. Food Protect*, 1997, núm 60, pp. 353–357).

It is difficult to isolate the bacteria when the water temperature is below 10°C (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed)", 1989, pp. 569–599; Høi L, Larsen JL, Dalsgaard I, & Dalsgaard A, "Occurrence of *Vibrio vulnificus* in Danish marine environments", *Appl. Environ. Microbiol*, 1998, núm. 64, pp. 7–13) and most cases, whether due to ingestion or injury, occur between the months of May and October (Oliver JD, "*Vibrio vulnificus*. In: Foodborne Bacterial Pathogens (Doyle MP, Ed)", 1989, pp. 569–599; Hlady WG, "*Vibrio* infections associated with raw oyster consumption in Florida, 1981–1994", *J. Food Protect*, núm. 60, 1997, pp. 353–357).

It has been speculated that this seasonal distribution of infections and isolation reflects the entry of *Vibrio vulnificus* into a viable but uncultivable state (Oliver JD, "Formation of viable but nonculturable cells. In: Starvation in Bacteria (Kjelleberg S, Ed)", 1993, *Plenum Press*, New York).

*Vibrio vulnificus* 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 vulnificus* infections. Based on surveillance data, from 1988 to 1996, the Centers for Disease Control and Prevention estimates that approximately 50 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 (Mead PS, Slutsker L, Dietz Y, McGaig LE, Bresee JS, Shapiro C, Griffin PM & Tauxe RV, "Food–related illness and death in the United States", *Emerg. Infect. Dis.*; núm. 5, 1999, pp. 607–625). Almost all infected people reported previous underlying chronic diseases, particularly liver disease (Shapiro RL, Altekruse S, Hutwagner R, Bishop R, Hammond S, Wilson B, Ray S, Thompson R, Tauxe V, Griffin PM & *Vibrio* working Group, "The role of Gulf Coast oysters harvested in warmer months in *Vibrio vulnificus* infections in the United States, 1988–1996", *J. Infect. Dis*, núm. 178, 1998, pp. 752–759).

Vibrios other than *Vibrio vulnificus* are estimated to cause approximately 5,000 foodborne infections per year in the United States of America (Mead PS, Slutsker L, Dietz Y, McGaig LE, Bresee JS, Shapiro C, Griffin PM & Tauxe RV, "Food–related illness and death in the United States", *Emerg. Infect. Dis.*, núm. 5, 1999, pp. 607–625) and *Vibrio parahaemolyticus* is generally considered to be the main cause of these infections (Hlady WG & Klontz KC, "The epidemiology of *Vibrio* infections in Florida, 1981–1993", *J. Infect. Dis.*, núm 173, 1996, pp. 1176–1183; Hlady WG, Mullen RC & Hopkin RS, "*Vibrio vulnificus* from raw oysters. Leading cause of reported deaths from foodborne illness in Florida", *J. Flo. Med. Assoc*, núm. 80, 1993, pp. 536–538). 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 (Honda T & Lida T, "The pathogenicity of *Vibrio parahaemolyticus* and the role of the thermostable direct hemolysin and related hemolysins", *Rev. Med. Microbiol*. núm. 4, 1993, pp. 106–113). 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 (Centers for Disease Control and Prevention, "Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters–Pacific Northwest, 1997", MMWR 47, 1998, pp. 457–462) concern and interest in this pathogen have increased (Centers for Disease Control and Prevention, "Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound–Connecticut, New Jersey and New York, 1998", MMWR 48, 1999, pp. 48–51; Daniels N, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond R, Thompson S, Wilson B, Bean N, Griffin P & Slutsker L, "*Vibrio parahaemolyticus* infections in the United States, 1973–1998" *J. Infect. Dis.*, núm. 181, 2000, pp. 1661–1666; Daniels NA, Ray B, Easton A, Marano N, Kahn E, McShan AL, Del Rosario L, Baldwin T, Kingsley MA, Puhr ND, Wells JG & Angulo EJ, "Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters. *JAMA*, 2000, núm 284, pp. 1541–1545). 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 (Okuda JM, Ishibashi ME, Hayashi E, Nishino T, Takeda Y, Mukhopadhyary AK, Garg S, Bhattacharya SK, Nair BG & Nishibuchi M, "Emergence of a unique O3:K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from southeast Asian travelers arriving in Japan, *J. Clin. Microbiol*, núm. 35, 1997, pp. 3150–3155). 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 (Daniels NA, Ray B, Easton A, Marano N, Kahn E, McShan AL, Del Rosario L, Baldwin T, Kingsley MA, Puhr ND, Wells JG & Angulo EJ, "Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters, *JAMA*, núm. 284, 2000, pp. 1541–1545).

One pathogen that can be transmitted by oysters is *Vibrio vulnificus*. Described in 1976 it was called "*Vibrio* lactose positive", later it was called *Beneckea vulnificus* and finally *Vibrio vulnificus*. 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*, 12 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 (Davalos MS, Natividad BJ, Vázquez SC & Quiñones RE. "Patógeno oportunista *Vibrio vulnificus*", *Revista Digital Universitaria*, vol. 6, núm. 4, 2005, pp. 2–10).

*Vibrio vulnificus* 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 (Poblete UR, Andresen HM, Pérez CC, Dougnac LA, Díaz PO, *et al*., "*Vibrio vulnificus*: una causa infrecuente de shock séptico", *Rev Méd Chile*, vol. 130, núm 7, pp. 787–791).

Because they are found in warm marine waters, people with open wounds can be exposed to *Vibrio vulnificus* through contact with marine waters, shellfish, and marine wildlife. There is no evidence of person–to–person transmission of *Vibrio vulnificus* 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 vulnificus* 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 vulnificus* infections are known to have liver disease or are immunosuppressed. On the other hand, healthy people have a lower risk of *Vibrio vulnificus* infection. Most *Vibrio vulnificus* diseases occur during the summer months (Oklahoma State Department of Health, *Vibrio vulnificus*. Hoja Informativa de Salud Pública, Internet: http://www.ok.gov/healsh2/documents/Vibrio%20wvulnificus%20–920Spanish.20051.pdf; Food Safety New Zealand, *Vibrio vulnificus*.

Internet: http://w1wuw.foodsafery govt.nzlelibrarylindustry/*Vibrio_vulnificus*–Science_Research.pdf).

Those responsible for the increase in the number of *Vibrio vulnificus* in fishery products at any given time are temperature, pH, salinity and the increase in organic matter, among others. *Vibrio vulnificus* 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 vulnificus* 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 vulnificus* 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 vulnificus* 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 vulnificus* has been implicated in human infections during the summer (Interstate Shellfish Sanitation Conference, *Vibrio vulnificus*. Hoja Informativa para los proveedores de Asistencia Médica.

Internet: http://www.issc.orglcliens_resources/Educasion/VvFactSheet.pdf).

The objective of the present study was to determine the prevalences of the *Vibrio vulnificus* 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 vulnificus* for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia.

## 2 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 vulnificus* 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 vulnificus*, thus constituting potential risk factors for the development of acute gastroenteritis, wound infection, primary septicemia and septicemia. secondary.

## 3 MATERIAL AND METHODS

**–Epistemological approach**

Quantitative approach, probabilistic approach or positivist approach (Hernández–Sampieri R, Fernández–Collado C, Baptista–Lucio MP, 2006, Research Methodology. Mexico: McGraw–Hill/Interamericana Editores, S.A. de C.V.).

**–Study design**

Descriptive, cross–sectional epizootiological study with no directionality and prospective temporality (Hernández–Ávila M, 2007, Epidemiology. Study Design and Analysis. Mexico: Editorial Médica Panamericana).

**–Study universe**

Representative sample selected from the total of two hundred samples of the 38 establishments that sell seafood for human consumption in the city of Telchac Puerto, Yucatan, Mexico. Said representative sample was taken in the period from July 1 to December 31, 2019.

**–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 (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and feces of food handlers", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**—Marine food.** Any product of animal origin from the sea that provides the human body with elements for its nutrition (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and in food handlers' feces", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**—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 (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and in feces of food handlers", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**—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 (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and in food handlers' feces", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**—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 5 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 cebiches (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and in food handlers' feces", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**—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) (Franco–Monsreal J & Flores–Abuxapqui JJ, "Prevalence of *Vibrio parahaemolyticus* in marine products and in food handlers' feces", Rev Lat–amer Microbiol, vol. 30, 1988, pp. 223–227).

**–Techniques and procedures**

A list of 38 establishments that specialize in the sale of marine food for human consumption was obtained. A first visit was made to each of the 38 establishments and compiled a list of 200 samples. The sampling scheme corresponding to the simple random sampling was used. The sample size was calculated

using the following statistician (Daniel WW. "Bioestadística. Base para el Análisis de las Ciencias de la Salud", *Editorial Limusa, México*, 1989, pp. 184 –185, 202–203):

$$n= NZ^2PQ / d^2(N–1) + Z^2PQ$$

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, 132 samples from the list of 200 of the 38 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 50 g; it was stored individually on a sterile polyethylene bag; it was stored in refrigeration and sent to the microbiology laboratory of the Centro de Investigaciones Regionales "Dr. Hideyo Noguchi" of the Universidad Autónoma de Yucatán.

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, 2019. For the homogenization and enrichment of each sample, as well as for the isolation and identification of the *Vibrio vulnificus* species, it was according to the methodology described in the eighth edition of the Food and Drug Administration Bacteriological Analytical Manual (Elliot EL, Kaysner CA, Jackson L & Tamplin ML, "*Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and other *Vibrio spp*. Chapter 9. In: Food and Drug Administration Bacteriological Analytical Manual, 8th ed. Editor: Merker RL. AOAC, MD, Arlington, Virginia, USA", 1988, pp. 9.01–9.27).

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–Haenszel ($x^2_{M–H}$) was used. The Epi Info Software for Windows, version 7.1.5.2, was used, for obtaining the values of the statistic's $x^2_{M–H}$ 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 (Cochran WG, "Some methods for strengthening the common $x^2$ tests", *Biometrics*, núm. 10, 1954, pp. 417–445): a) when $n > 40$ use the $x^2_{M–H}$ test; b) when $20 \leq n \leq 40$ use the $x^2_{M–H}$ 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.

$$x^2_{M-H} = \Sigma ( |O - E| - ½)^2 / E$$

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

The cornfield estimation interval was built at the 95% confidence level for the percentage in the seabed population with *Vibrio vulnificus*. Said estimate interval was built using the following statistician (Daniel WW, "Bioestadística. Base para el Análisis de las Ciencias de la Salud". *Editorial Limusa, México*, 1989, pp. 184–185, 202–203):

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

Where:
p= proportion of elements in the sample that possesses the interest characteristic; Z= level of confidence; and σp= standard error; and 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; and n= sample size.
The Cornfield estimation interval at the 95% confidence level for the percentage in the seabed population with *Vibrio vulnificus* was 13.56% ≤ P ≤ 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. The species investigated in the present work belongs to Group 6 (negative production of arginine dehydrolase and positive disarrangement of lysine).

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

| | **Group 1** | | **Group 2** | **Group 3** | **Group 4** | **Group 5** | | | **Group 6** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Key differential tests** | **Vibrio cholerae** | **Vibrio mimicus** | **Vibrio metschnikovii** | **Vibrio cincinnatiensis** | **Vibrio hollisae** | **Vibrio damsela** | **Vibrio fluvialis** | **Vibrio furnissii** | **Vibrio alginolyticus** | **Vibrio parahaemolyticus** | **Vibrio vulnificus** | **Vibrio carchariae** |
| 1. Growth on Nutrient Agar with 0% NaCl | + | + | | | | | | | | | | |
| 2. Growth on Nutrient Agar with 1% de NaCl | + | + | | | | | | | | | | |
| 3. Oxidase test | | | – | | | | | | | | | |
| 4. Reduction of nitrates ($NO_3$) to nitrites ($NO_2$) | | | – | | | | | | | | | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5. Myo–inositol fermentation | | | | + | | | | | | | | |
| 6. Production of arginine dehydrolase | | | | | – | + | + | + | – | – | – | – |
| 7. Lysine decarboxylation | | | | | – | | | | + | + | + | + |
| 8. Ornithine Decarboxylation | | | | | – | | | | | | | |

Source. Kelly MT, Hickman–Brenner FW, Farmer JJ III. 1991, "*Vibrio*: In Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ (Editors). Manual of Clinical Microbiology (5ª Ed.). Washington, D.C.", *American Society for Microbiology*, 1991, p. 389.

**–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 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.

## 4 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, molluscs and fish) and 19 studied species (shrimp, crab, jaiba, squid, snail, oyster, octopus, abadejo, bulkine, czon, crowned, corvine, chihua, mere, pramp, snapper, picuda, blonde and saw).

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

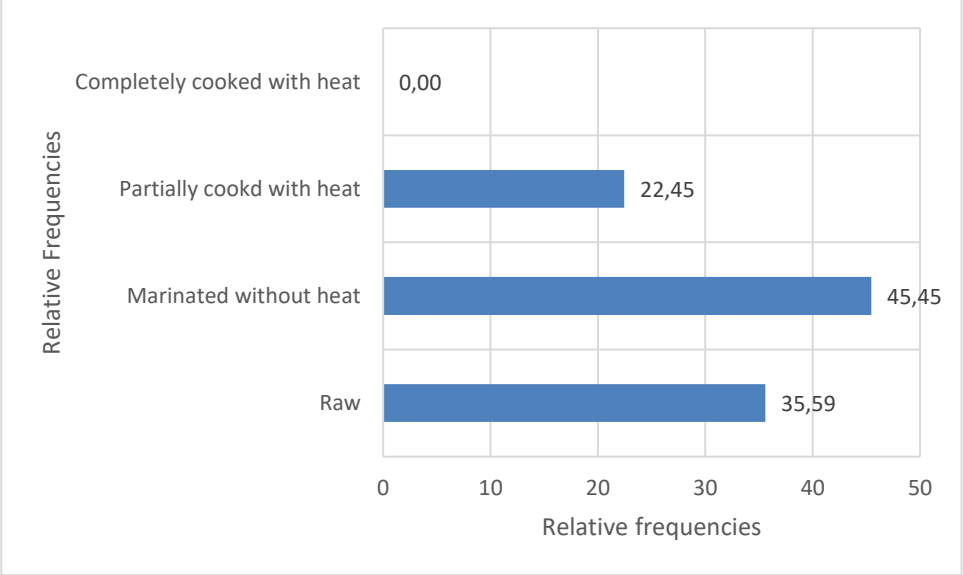
Table 2. Absolute and relative frequencies of marine food by prevalence *Vibrio vulnificus* according to preparation methods.

| **Preparation methods** | **Prevalences** | **Totals** |
|---|---|---|
| | ***Vibrio vulnificus*** | |
| 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

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

Figure 1. Relative frequencies of marine foods by prevalences of *Vibrio vulnificus* according to preparation methods.




Source. Table 2

In 37 (28.03%) samples an equal number of strains whose biochemical characteristics corresponded to *Vibrio vulnificus* were isolated. The prevalences obtained in raw, 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 $x^2_{M-H}$ 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: $x^2_{M-H}$ ($\alpha$= 0.0500, gl= 1) > 3.8416; $p$< 0.0500.

## 5 DISCUSSION

With respect to the *Vibrio vulnificus* 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.

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 vulnificus* (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 in the 13 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.

## 6 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 vulnificus* species for the development of acute gastroenteritis, wound infection, primary septicemia and secondary septicemia.

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