Microbiological hazards associated with bivalve mollusc consumption

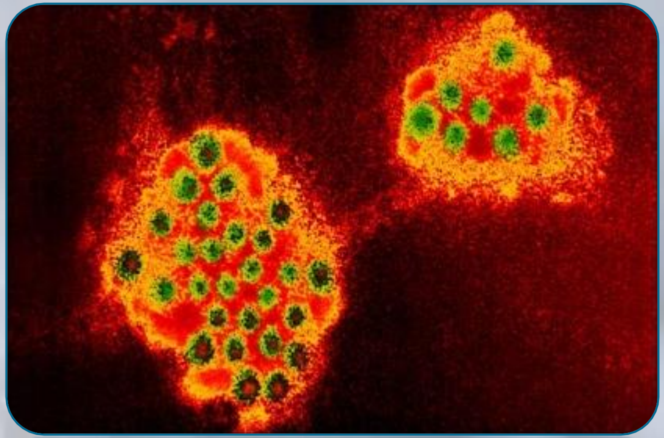
James Lowther & Craig Baker-Austin

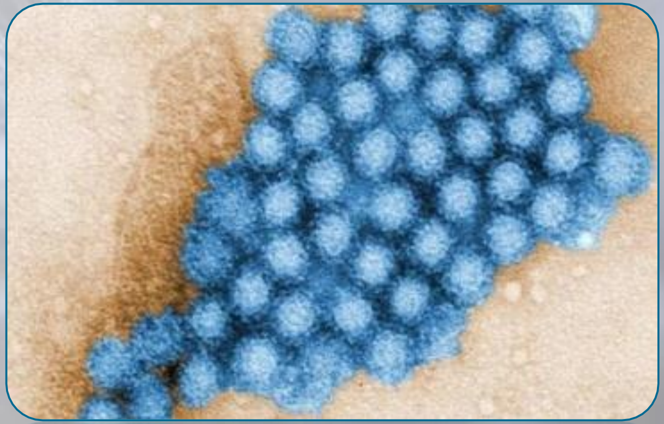
Shellfish and disease risk

Shellfish an established route of transmission for a range of important human pathogens and other agents of illness. First outbreaks linked to shellfish consumption emerged in the 19th Century.

Filter feeding bivalves (oysters, mussels, clams etc.) very efficient at concentrating microbes, algal toxins and other chemicals. Bivalve shellfish can concentrate microbial pathogens >100 times compared with overlying waters.

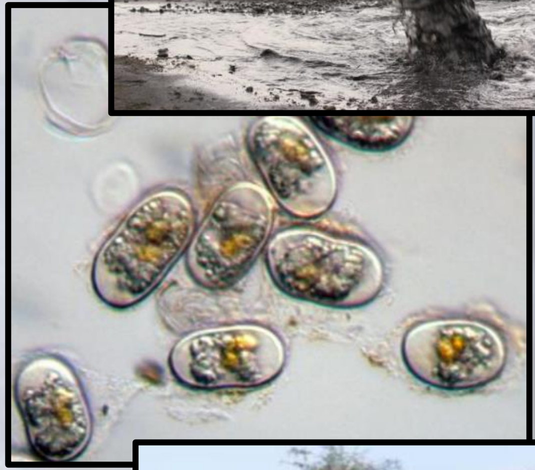
Outbreaks can be very large: Hepatitis A outbreak in China in 1988 due to contamination of clams was responsible for almost 300,000 cases – one of the largest foodborne outbreaks ever reported.





Types of hazard associated with bivalve shellfish

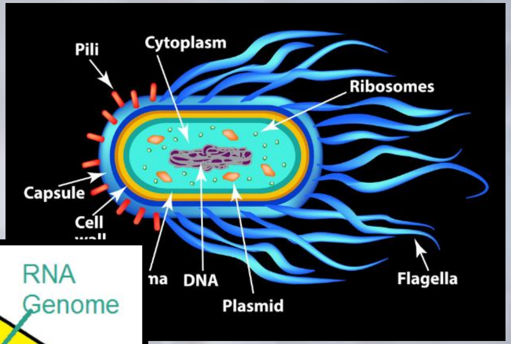
TYPE OF HAZARD	SOURCES	GUIDANCE
CHEMICAL e.g. pesticides, heavy metals	Agricultural run-off, industrial discharges etc.	Codex Alimentarius, General Standard for Contaminants and Toxins in Feed and Food, 2009
BIOTOXINS i.e. toxic chemicals produced by marine microalgae	Naturally occurring in seawater, blooms	Assessment and management of biotoxin risks in bivalve molluscs, FAO Technical Paper, 2011
MICROBIOLOGICAL i.e. pathogenic bacteria, viruses, parasites	Human sewage, animal faeces, some naturally occurring in seawater	FAO/WHO Technical Guidance for the Development of Sanitation Programmes

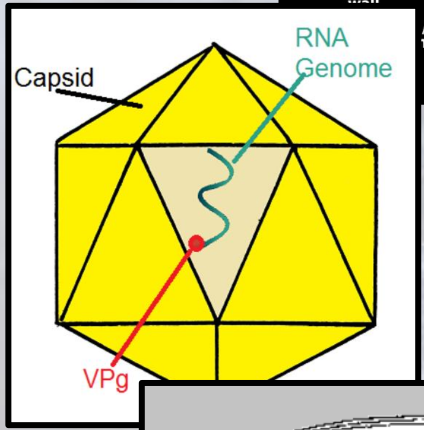

Microbiological hazards

Numerous microbiological pathogens potentially linked to shellfish consumption

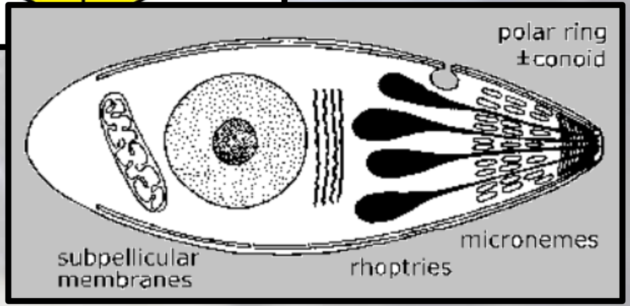
BACTERIA — *Salmonella* spp., *Vibrio* spp., *Campylobacter* spp., *Listeria monocytogenes*



VIRUSES — Norovirus, hepatitis A virus, sapovirus, hepatitis E virus



PARASITES — *Giardia intestinalis*, *Cryptosporidium parvum*, *Microsporidia*



Microbiological hazards

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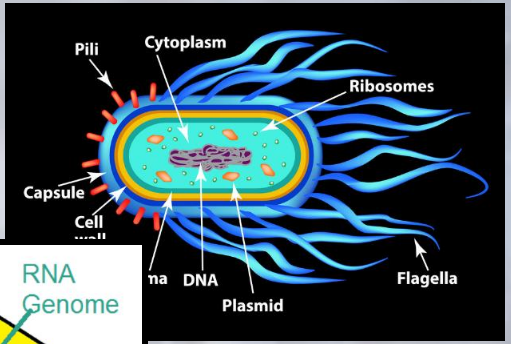
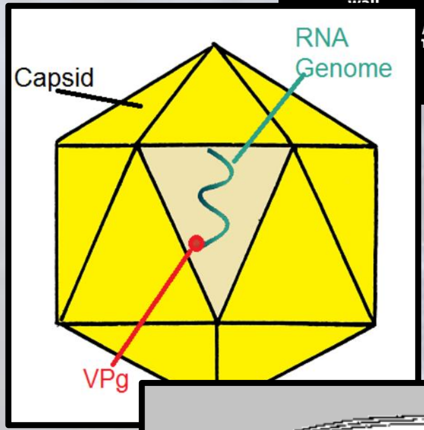
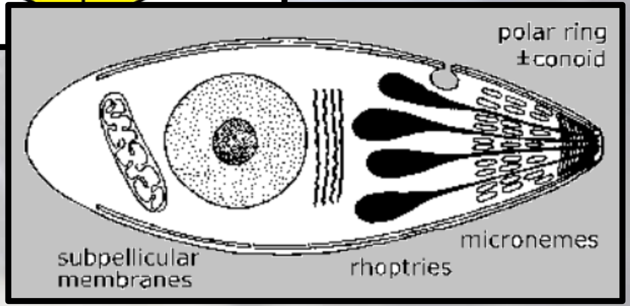
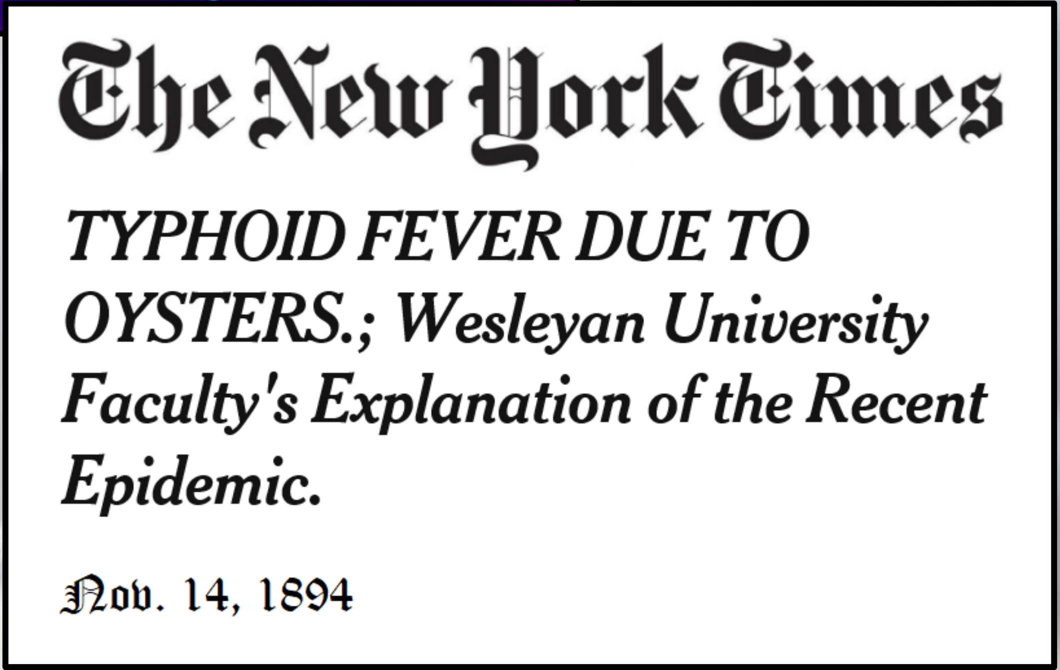






Salmonella enterica serovars Typhi & Paratyphi

- Gram negative bacterium
- Causes enteric fever (severe illness)
- Transmitted in human faeces
- First recorded outbreak due to shellfish consumption in **1894**



The New York Times

TYPHOID FEVER DUE TO OYSTERS.; Wesleyan University Faculty's Explanation of the Recent Epidemic.

Nov. 14, 1894

Vibrio parahaemolyticus and *V. vulnificus*

- Gram negative bacteria

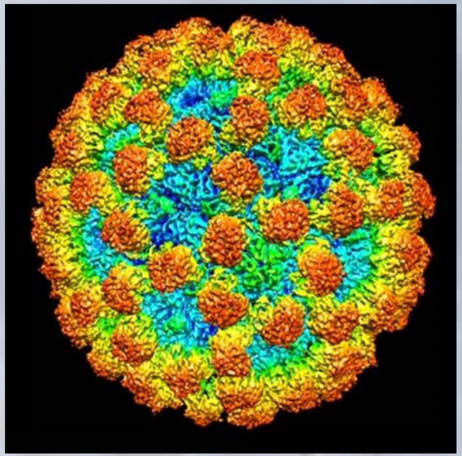
- Cause gastroenteritis (Vp; mild illness) or sepsis (Vv; severe illness with high mortality in susceptible cases)

- Naturally occurring in marine environment; associated with low salinity, high temperature coastal waters

- Commonest shellfish–related pathogen in e.g. USA (Vp)

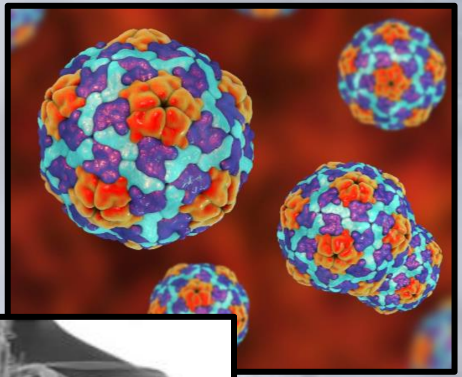
Norovirus

- Single stranded RNA virus
- Causes gastroenteritis (normally mild illness)
- Transmitted in human faeces
- Widespread worldwide
- Commonest shellfish-related pathogen in e.g. Europe
- Highly seasonal occurrence **<u>in some regions</u>**



Hepatitis A virus

- Single stranded RNA virus
- Causes hepatitis (moderate illness)
- Transmitted in human faeces
- Frequency in human populations varies widely across the globe



Shellfish-related pathogens in Latin America and the Caribbean

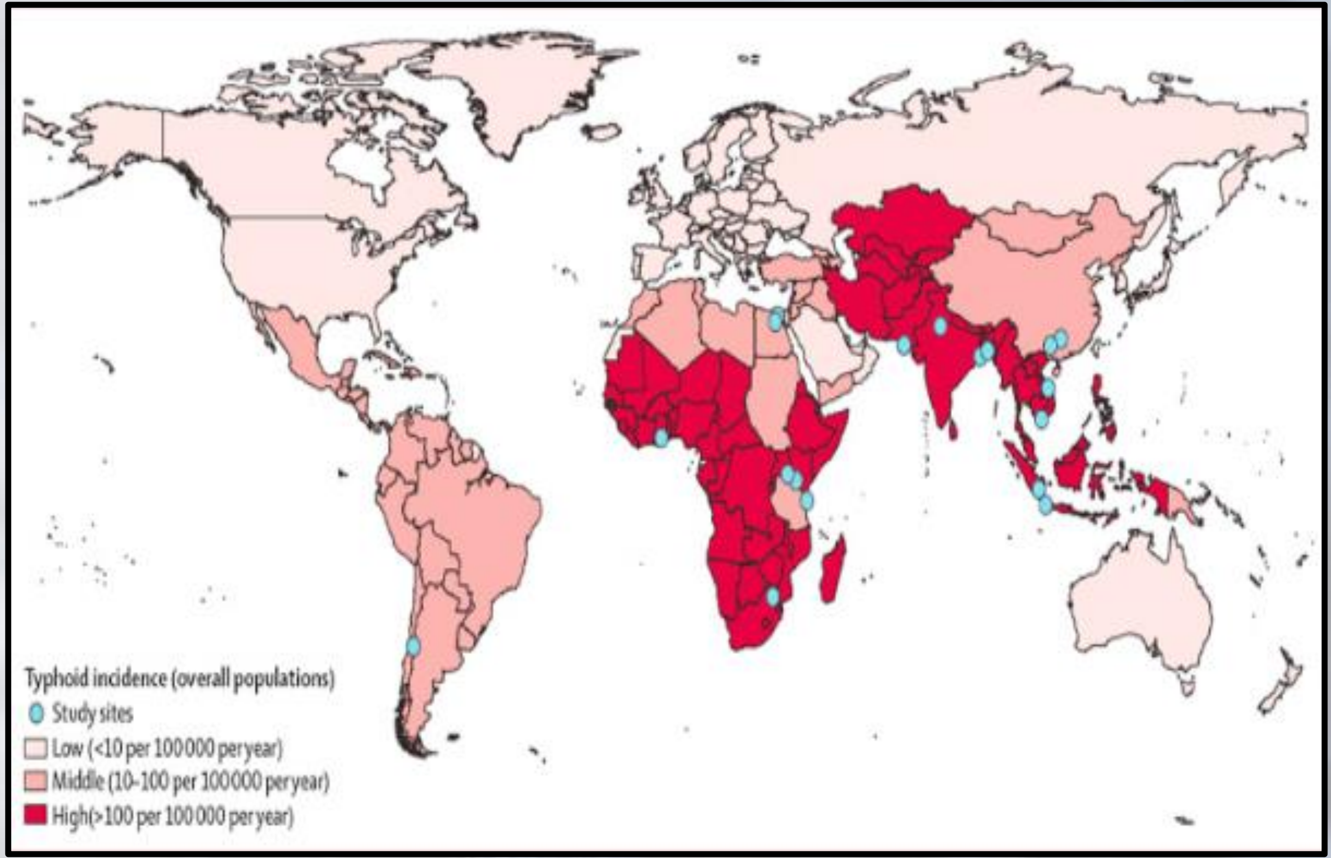
- All major shellfish-related pathogens found in the Latin American and Caribbean general population

- Some reports of pathogen detection in shellfish samples

- So far a small number of reports of shellfish-related outbreaks

Salmonella spp.

Mid-level incidence of typhoid fever across Latin America and the Caribbean



Salmonella spp.

Small number of reports of *Salmonella spp.* isolated from shellfish in Latin America and The Caribbean

Int J Environ Health Res. 2007 Aug;17(4):259-69. doi: 10.1080/09603120701372169.

Pathogenic bacteria associated with oysters (Crassostrea brasiliana) and estuarine water along the south coast of Brazil

Christiane A Ristori [1], Sebastião T Iaria, Dilma S Gelli, Irma N G Rivera

Affiliations − collapse

Affiliation

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São Paulo, Brazil.

PMID: 17613090 DOI: 10.1080/09603120701372169

Epidemiol. Infect. (1999), **123**, 241–250. Printed in the United Kingdom

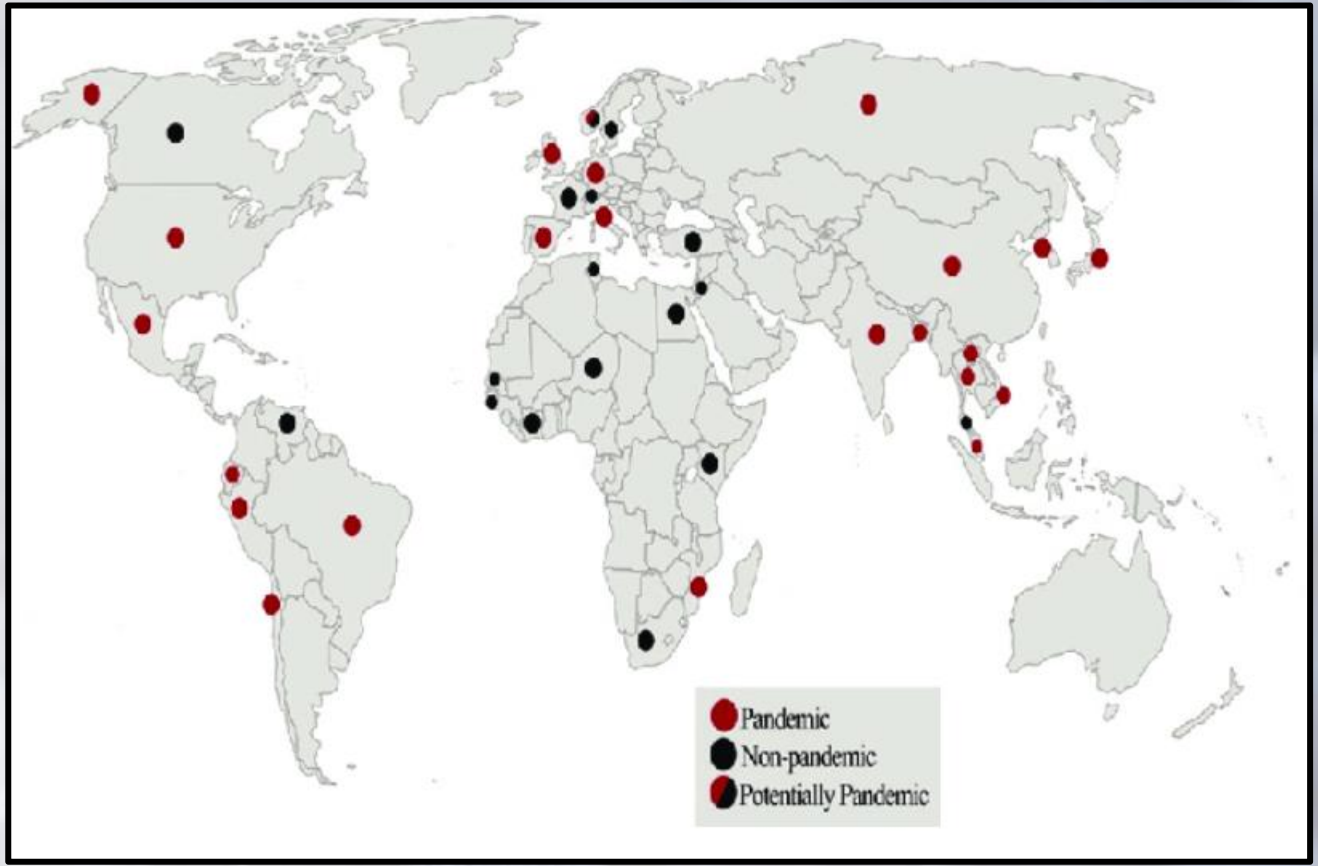
Microbial quality of oysters sold in Western Trinidad and potential health risk to consumers

F. S. RAMPERSAD[1], S. LALOO[1], A. LA BORDE[1], K. MAHARAJ[1], L. SOOKHAI[1], J. TEELUCKSINGH[1], S. REID[1], L. MCDOUGALL[1] AND A. A. ADESIYUN[2]*

[1] *School of Medicine, Faculty of Medical Sciences, University of the West Indies, St Augustine, Trinidad and Tobago*
[2] *School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St Augustine, Trinidad and Tobago*

Vibrio spp.

Vibrio parahaemolyticus recorded in multiple Latin American Countries



Vibrio spp.

Significant reports of detection of *Vibrio* spp. in shellfish in Latin America and the Caribbean

Revista da Sociedade Brasileira de Medicina Tropical 47(3):327-333, May-Jun, 2014
http://dx.doi.org/10.1590/0037-8682-0069-2014
Major Article

Occurrence of potentially pathogenic *Vibrio* in oysters (*Crassostrea gigas*) and waters from bivalve mollusk cultivations in the South Bay of Santa Catarina

Roberta Juliano Ramos[1], *Letícia Adélia Miotto*[1], *Marília Miotto*[1], *Nelson Silveira Junior*[2], *Andréia Cirolini*[1], *Helen Silvestre da Silva*[1], *Dália dos Prazeres Rodrigues*[3] *and Cleide Rosana Werneck Vieira*[1]

[1]. Laboratório de Microbiologia de Alimentos, Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Santa Catarina, Florianópolis, SC. [2]. Associação de Maricultores do Sul da Ilha, Florianópolis, SC. [3]. Laboratório de Enterobactérias, Instituto Oswaldo Cruz, Rio de Janeiro, RJ.

> Environ Microbiol. 2006 Apr;8(4):675-83. doi: 10.1111/j.1462-2920.2005.00946.x.

Vibrio parahaemolyticus in shellfish and clinical samples during two large epidemics of diarrhoea in southern Chile

Loreto Fuenzalida [1], Cristina Hernández, Jessica Toro, M Luisa Rioseco, Jaime Romero, Romilio T Espejo

Affiliations — collapse

Affiliation

1 Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile.

PMID: 16584479 DOI: 10.1111/j.1462-2920.2005.00946.x

www.medigraphic.org.mx

RESPYN Revista Salud Pública y Nutrición

Volumen 11 No. 1
Enero-Marzo 2010
Salus cum propositum vitae

ESTUDIO PILOTO PARA EL AISLAMIENTO DE *VIBRIO* SPP EN OSTRAS (*CRASSOSTREA RHIZOPHORAE*) CAPTURADAS EN LA CIÉNAGA DE LA VIRGEN, CARTAGENA, COLOMBIA

Lersy López, Ganiveth Manjarrez, Lilibeth Herrera, Ana Montes, Yuranis Olascuaga y Rolando Ortega
Grupo de Investigaciones Biomédicas
Instituto de Investigaciones Alberto Montealegre (IDIBAM)
Universidad de San Buenaventura (Cartagena, Colombia)
E-mail: rjortega@enred.com

Zootecnia Trop., 26(4): 505-513. 2008

Prevalencia de *Staphylococcus aureus*, *Vibrio* spp. y enterobacterias en carne de pepitona, *Arca zebra*, comercializada en Cumaná, Venezuela

Daniel Muñoz[1*], Crucita Graü de Marín[2], Carlos Martínez[1], Hilda Marval[2] y Aracelis Zerpa[2]

[1] Departamento de Biología, Escuela de Ciencias, Universidad de Oriente, Núcleo de Sucre. Cumaná, Sucre. Venezuela. *Correo electrónico: d_josem77@hotmail.com
[2] Instituto Nacional de Investigaciones Agrícolas, Centro de Investigaciones Agrícolas de los estados Sucre/Nueva Esparta. Laboratorio de Microbiología de Alimentos. Cumaná, Sucre. Venezuela.

Norovirus

Norovirus a common cause of gastroenteritis in Latin America and the Caribbean

Brief Report

High Prevalence and Increased Severity of Norovirus Mixed Infections Among Children 12–24 Months of Age Living in the Suburban Areas of Lima, Peru

Mara Zambruni,[1] Giannina Luna,[2] Maria Silva,[2] Daniel G. Bausch,[2,3] Fulton P. Rivera,[4] Grace Velapatino,[4] Miguel Campos,[5] Elsa Chea-Woo,[4] Nelly Baiocchi,[4] Thomas G. Cleary,[6] and Theresa J. Ochoa[4,6]
[1]Department of Pediatrics, Division of Infectious Diseases, University of Texas, Houston; [2]US Naval Medical Research Unit No. 6, Lima, Peru; [3]Tulane School of Public Health and Tropical Medicine, New Orleans, Louisiana; Departaments of [4]Pediatrics; [5]Mathematics, Universidad Peruana Cayetano Heredia, Lima, Peru; and [6]Center for Infectious Diseases, University of Texas School of Public Health, Houston

Corresponding Author: Theresa J. Ochoa, MD, Department of Pediatrics, Instituto de Medicina Tropical "Alexander von Humboldt," Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin de Porras, Lima 31, Peru. E-mail: Theresa.J.Ochoa@uth.tmc.edu

Received July 15, 2014; accepted January 3, 2015; electronically published February 4, 2015.

Journal of
Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Norovirus molecular detection in Uruguayan sewage samples reveals a high genetic diversity and GII.4 variant replacement along time

M. Victoria[1], L.F.L. Tort[1], A. Lizasoain[1], M. García[1], M. Castells[1], M. Berois[2], M. Divizia[3], J.P.G. Leite[4], M.P. Miagostovich[4], J. Cristina[5] and R. Colina[1]

1 Laboratory of Molecular Virology, Department of Biological Sciences, Regional Norte, CENUR Litoral Norte, Universidad de la República, Salto, Uruguay
2 Virology Section, School of Sciences, Universidad de la República, Montevideo, Uruguay
3 Laboratory of Environmental Virology, Department of Experimental Medicine and Surgery, Tor Vergata University, Rome, Italy
4 Laboratory of Comparative and Environmental Virology, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil
5 Laboratory of Molecular Virology, Nuclear Investigation Center, School of Sciences, Universidad de la República, Montevideo, Uruguay

Brazilian Journal of Medical and Biological Research (2004) 37: 321-326
ISSN 0100-879X

Noroviruses associated with acute gastroenteritis in a children's day care facility in Rio de Janeiro, Brazil

C.I. Gallimore[1], M.A.B. Barreiros[2,4], D.W.G. Brown[1], J.P. Nascimento[3] and J.P.G. Leite[4]

[1]Enteric Virus Unit, Enteric, Respiratory and Neurological Virus Laboratory, Specialist and Reference Microbiology Division, Health Protection Agency, Colindale, London, UK
[2]UNIVALI, Itajaí, SC, Brasil
[3]Departamento de Desenvolvimento Tecnológico, Bio-Manguinhos, Fiocruz, and
[4]Laboratório de Virologia Comparada, DV, Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ, Brasil

Clinical Infectious Diseases

MAJOR ARTICLE

Incidence and Clinical Profile of Norovirus Disease in Guatemala, 2008–2013

Marieke Bierhoff,[1,2] Wences Arvelo,[1] Alejandra Estevez,[3] Joe Bryan,[1] John P. McCracken,[3] María Reneé López,[3] Beatriz López,[3] Umesh D. Parashar,[1] Kim A. Lindblade,[1] and Aron J. Hall[1]
[1]Centers for Disease Control and Prevention, Atlanta, Georgia; [2]VU Medical Center, Amsterdam, The Netherlands; and [3]Universidad del Valle de Guatemala, Guatemala City

Norovirus

Small number of reports of norovirus detection in shellfish from Latin America and the Caribbean or consumers thereof

ELSEVIER

Marine Pollution Bulletin
Volume 157, August 2020, 111315

Human norovirus detection in bivalve shellfish in Brazil and evaluation of viral infectivity using PMA treatment

Sylvia Kahwage Sarmento [a], Caroline Rezende Guerra [b], Fábio Correia Malta [a], Ricardo Coutinho [b], Marize Pereira Miagostovich [a], Tulio Machado Fumian [a]

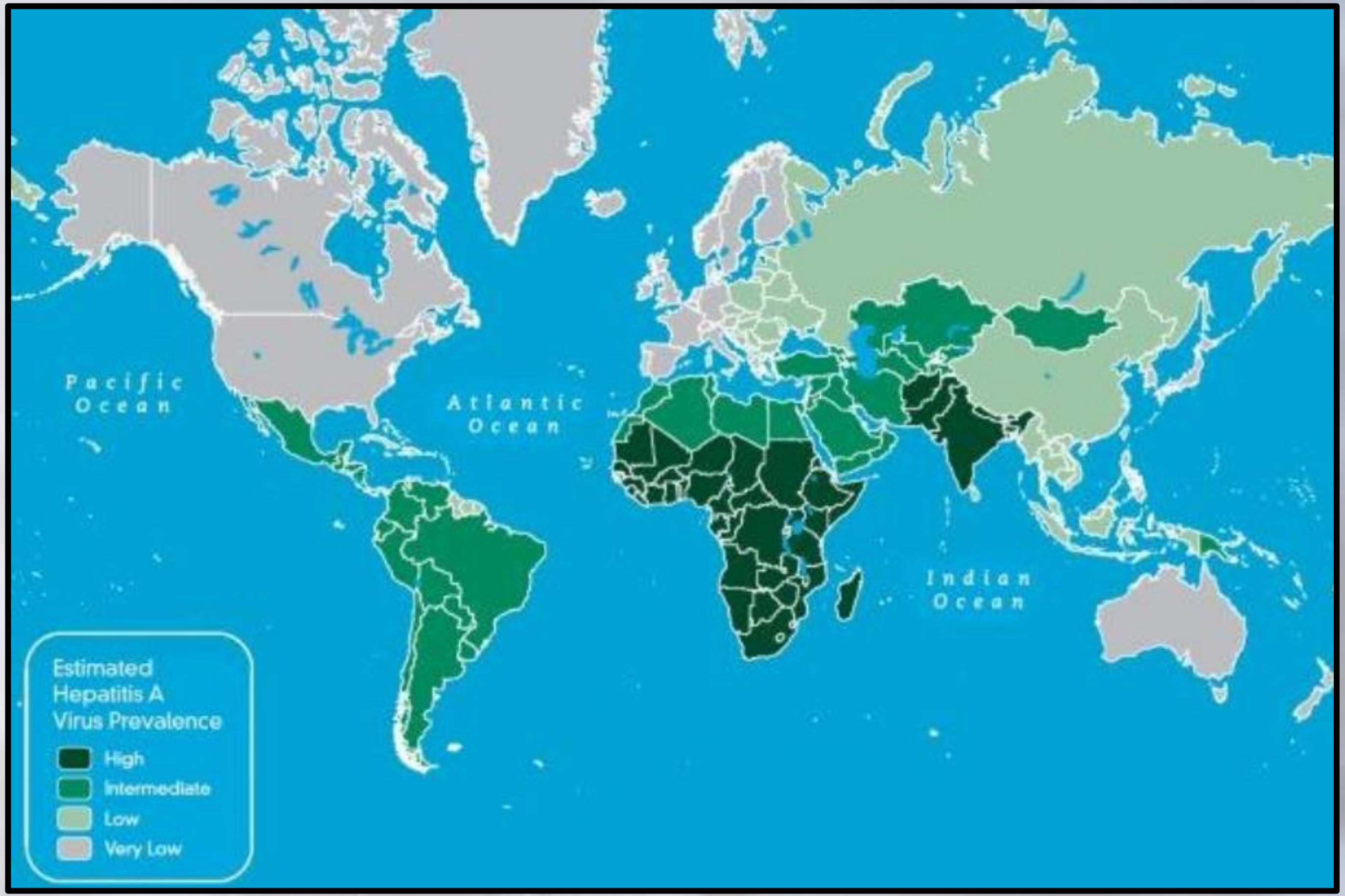
[a] Laboratório de Virologia Comparada e Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ CEP 21045-900, Brazil
[b] Laboratório de Genética Marinha, Departamento de Biote
Almirante Paulo Moreira (IEAPM), Arraial do Cabo , RJ CEP

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Multistate Outbreak of Gastrointestinal Illnesses Linked to Oysters Imported from Mexico

Hepatitis A virus

Intermediate prevalence of HAV in most parts of Latin America and the Caribbean



Hepatitis A virus

HAV occasionally reported in shellfish from Latin America and The Caribbean – some large outbreaks in importer countries have been reported

Journal of Food Protection, Vol. 66, No. 3, 2003, Pages 507–511

Research Note

Hepatitis A Virus Detection in Oysters (*Crassostrea gigas*) in Santa Catarina State, Brazil, by Reverse Transcription–Polymerase Chain Reaction

C. COELHO,[1,2,4] A. P. HEINERT,[1,2] C. M. O. SIMÕES,[1,3] AND C. R. M. BARARDI[1,2]*

[1]*Laboratório de Virologia Aplicada,* [2]*Departamento de Microbiologia e Parasitologia, Centro de Ciências Biológicas, and* [3]*Departam Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina 88040-900, Florianópolis, Santa Catarin and* [4]*Departamento de Farmácia, Centro de Ciências da Saúde, Universidade do Vale de Itajaí, Santa Catarina, Brazil*

MS 01-424: Received 15 April 2002/Accepted 18 September 2002

JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2002, p. 4148–4155 Vol. 40, No. 11
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.11.4148–4155.2002

Molecular Characterization of Hepatitis A Virus Isolates from a Transcontinental Shellfish-Borne Outbreak

Glòria Sánchez,[1] Rosa M. Pintó,[1] Hermelinda Vanaclocha,[2] and Albert Bosch[1]*

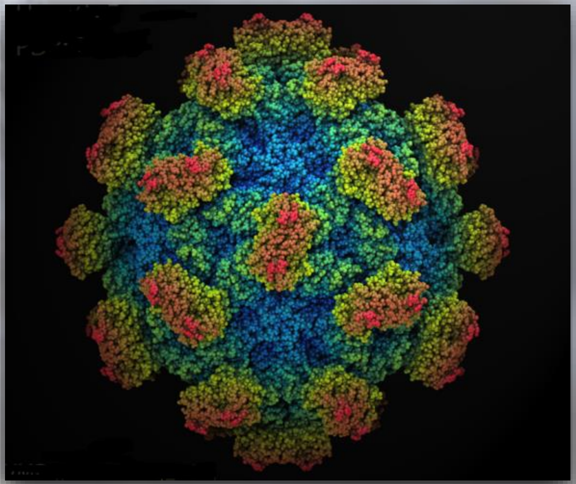
Department of Microbiology, University of Barcelona, Barcelona,[1] *and Department of Epidemiology, Generalitat Valenciana, Valencia,*[2] *Spain*

Received 24 April 2002/Returned for modification 15 June 2002/Accepted 5 August 2002

One hundred eighty-four serologically confirmed cases of hepatitis A were reported in eastern Spain in 1999. A matched case-control study implicated imported coquina clams complying with European Union shellfish standards as the source of infection; this implication was confirmed by the detection by reverse transcription-PCR of hepatitis A virus (HAV) RNA in shellfish samples. In spite of the recognized low variability of HAV, genetic characterization of the complete capsid region of virus isolates from patient serum samples revealed the existence of both synonymous and nonsynonymous variants. Two antigenic variants were detected, one in a discontinuous epitope defined by monoclonal antibody K3-4C8 and a second in a linear VP1 epitope of the virus. In spite of these antigenic variants, all isolates were assigned to genotype IB, providing further evidence that the outbreak originated from a common source, although multiple strains were likely to be involved.

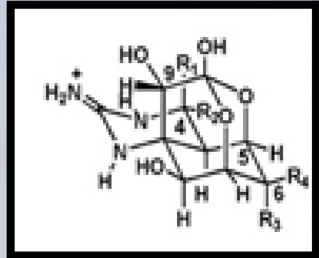
Emerging risks – Hepatitis E Virus

- Hepatitis E virus (HEV) an important and emerging zoonotic pathogen:-
  - Primary host pigs (also deer etc.)
  - 20,000,000 human infections worldwide in 2005
  - 44,000 deaths in 2015
  - Large increase in reported infections in last decade
- HAV spreads via faeco-oral route, shed in both faeces and urine
- Infection usually occurs through contact with faecally-contaminated water or consumption of undercooked or raw meat products.
- HEV often detected in bivalves. Potential route of human transmission?



Emerging risks - Tetrodotoxin

- Tetrodotoxin (TTX) is a potent neurotoxin, normally associated with pufferfish poisoning (Japan)
- Produced by marine bacteria –pathways of TTX production not well understood
- First detection of TTX in shellfish (UK mussels) in 2013-2014
- Method development at Cefas and UK national surveillance in 2016-2017
- Subsequent studies have detected TTX in bivalves across Europe and also in Asia
- EFSA opinion on risks recently published



RAPID COMMUNICATIONS

Detection of the pufferfish toxin tetrodotoxin in European bivalves, England, 2013 to 2014

A D Turner (andrew.turner@cefas.co.uk)[1], A Powell[1], A Schofield[1,2], D N Lees[1], C Baker-Austin[1]
1. Food Safety Group, Centre for Environment Fisheries and Aquaculture Science, Weymouth, Dorset, United Kin
2. Department of Chemistry, University of Hull, Hull, United Kingdom

TURNER ET AL.: JOURNAL OF AOAC INTERNATIONAL VOL. 100, NO. 5, 2017 1

FOOD CHEMICAL CONTAMINANTS

Development and Single-Laboratory Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for Quantitation of Tetrodotoxin in Mussels and Oysters

ANDREW D. TURNER
Centre for Environment, Fisheries, and Aquaculture Science, Barrack Rd, The Nothe, Weymouth, Dorset, DT4 8UB, United Kingdom
MICHAEL J. BOUNDY
Cawthron Institute, 98 Halifax St East, Nelson 7010, Private Bag 2, Nelson 7042, New Zealand
MONIKA DHANJI RAPKOVA
Centre for Environment, Fisheries, and Aquaculture Science, Barrack Rd, The Nothe, Weymouth, Dorset, DT4 8UB,

marine drugs MDPI

Article

Detection of Tetrodotoxin Shellfish Poisoning (TSP) Toxins and Causative Factors in Bivalve Molluscs from the UK

Andrew D. Turner [1,*], Monika Dhanji-Rapkova [1], Lewis Coates [1], Lesley Bickerstaff [1], Steve Milligan [1], Alison O'Neill [1], Dermot Faulkner [2], Hugh McEneny [2], Craig Baker-Austin [1], David N. Lees [1] and Myriam Algoet [1]

Changing risk factors increasing shellfish risk

1. **Rapidly ageing population**. Percentage of people aged >65 has increased steadily in the last 3 decades. Age a major risk factor for more aggressive/systemic *Vibrio* infections, such as *V. vulnificus* and *V. cholerae* (non-O1/O139).

2. **Underlying conditions**. The number of people with underlying risk factors are increasing steadily – these include liver dysfunction, diabetes and compromised immune systems.

3. **Exposure**. More people living by, and in direct contact with water.

4. **Shellfish consumption.** Globally there is increasing consumption and international trade of raw/lightly cooked shellfish.

Greater international trade in shellfish = greater human health risks

Risk assessment

- As part of the Growing Area Risk Profile, the relative risk posed by different hazards should be assessed based on relevant factors e.g.:-
  - Pollution sources affecting the growing area (human sewage, agricultural, industrial etc.)
  - Seasonality of harvest
  - Water temperature and salinity
  - Method of processing of finished shellfish
  - Epidemiological data on pathogens in population – **<u>NOTE absence of data does not necessarily imply absence of risk</u>**

PRINCIPAL SOURCE AND FACTOR CONSIDERATIONS FOR THE MAJOR HAZARD GROUPS

HUMAN ENTERIC PATHOGENS	ANIMAL ENTERIC PATHOGENS	MARINE VIBRIOS	BIOTOXINS	CHEMICAL CONTAMINANTS
Continous sewage discharges	Farm animal polpulations	Concentration of potentially pathogennic species	Occurrence of potentially toxic phytoplankton	Industrial effluents
Intermittent sewage discharges	Animal waste storage locations	Proportion of pathogenic strains	Occurrence of biotoxins in bivalves	Landfill sites
Private sewage discharges	Slurry spreading locations	Seawater temperature	Seawater temperature	Mining
Direct defecation to land	Terrestrial wild animal populations	Seawater salinity	Seawater salinity	Shipping & boating
Sewage sludge spreading	Marine wild animal populations			Marine waste disposal sites
Rainfall-dependent discharges (or run-off)	Wild bird populations			Geological sources
Shipping & boating	Rainfall mediated run-off			Contaminated sediments

Hydrography

Hazard Survey

- Depending on the results of the Growing Area Risk Profile, it may be necessary to include a hazard survey as part of the Growing Area Assessment

- Hazard survey involves testing samples of shellfish (or water) for pathogens

- Methods can be complex and require specialist equipment

Methods

Norovirus and Hepatitis A virus	ISO 15216-1	Quantification using real-time RT-PCR
Salmonella spp.	ISO 6579-1	Detection by growth on selective bacteriological media – confirmation using biochemical/serological tests
Vibrio spp.	ISO 21872-1	Detection by growth on selective bacteriological media – confirmation using biochemical/PCR tests

Method for Norovirus and Hepatitis A virus (ISO 15216-1)

- Multi-stage procedure including a comprehensive suite of controls
- Digestive tissues dissected from the shellfish, then virus extracted using a digestion with Proteinase K solution
- Viral RNA purified from digested sample using guanidine isothiocyanate to denature virus proteins and magnetic silica beads as RNA-binding matrix
- Detection of target viruses using TaqMan real-time RT-PCR (one-step) with virus-specific primer and probe sequences
- Negative and positive controls, standard curves for quantification and controls for extraction efficiency and RT-PCR inhibition included

Hazard Survey

- Depending on the results of the Growing Area Risk Profile, it may be necessary to include a hazard survey as part of the Growing Area Assessment

- Hazard survey involves testing samples of shellfish (or water) for pathogens

- Methods can be complex and require specialist equipment

- Laboratory accreditation to ISO 17025 desirable

Summary

- Wide variety of chemical, biotoxin and microbiological hazards associated with shellfish consumption

- Microbiological hazards include bacteria (*Salmonella, Vibrio*), viruses (norovirus, hepatitis A virus), parasites

- Emerging and increasing risks

- Risks posed by different hazards may depend on the characteristics of the growing area

- Specific testing for hazards may require specialist laboratories