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Organization of the  
United Nations



**Cefas**

# **VIRTUAL REGIONAL WORKSHOP ON BIVALVE MOLLUSCS SANITATION**

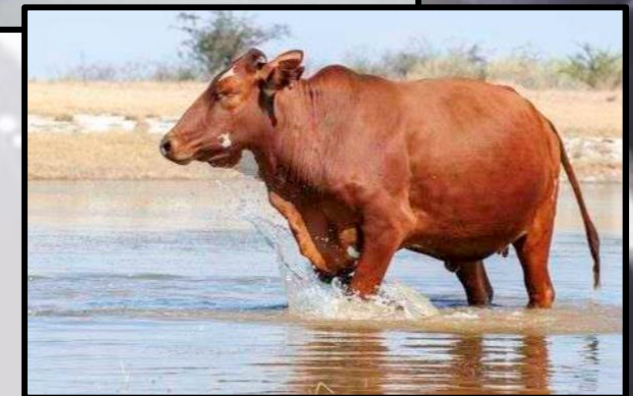
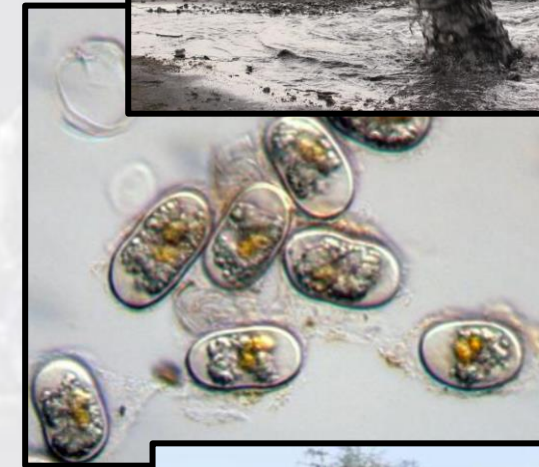
9, 10, 11 December 2020

**Overview of hazards associated with  
bivalve mollusc consumption: Hazard  
characterisation and risk assessment**

James Lowther

# Types of hazard associated with bivalve shellfish

TYPE OF HAZARD	SOURCES	GUIDANCE
<b>CHEMICAL</b> e.g. pesticides, heavy metals	Agricultural run-off, industrial discharges etc.	Codex Alimentarius, General Standard for Contaminants and Toxins in Feed and Food, 2009
<b>BIOTOXINS</b> 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
<b>MICROBIOLOGICAL</b> 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 – <b><u>this workshop</u></b>

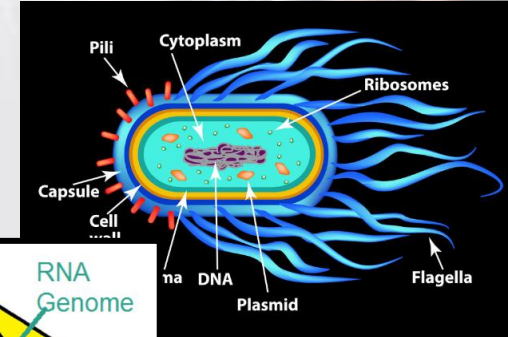


# Microbiological hazards

Numerous microbiological pathogens potentially linked to shellfish consumption

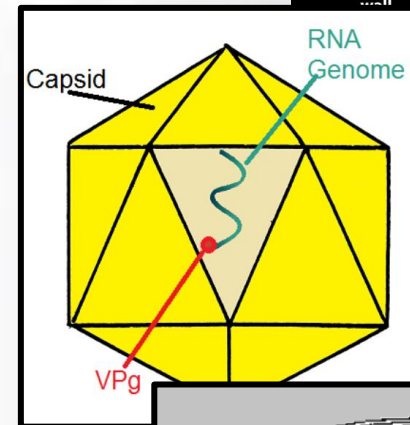
## BACTERIA

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



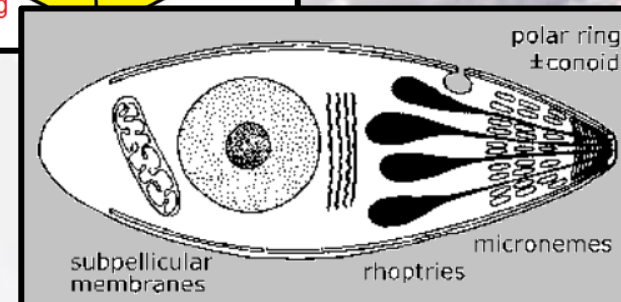
## VIRUSES

Norovirus, hepatitis A virus,  
sapovirus, hepatitis E virus



## PARASITES

*Giardia intestinalis*,  
*Cryptosporidium parvum*,  
*Microsporidia*

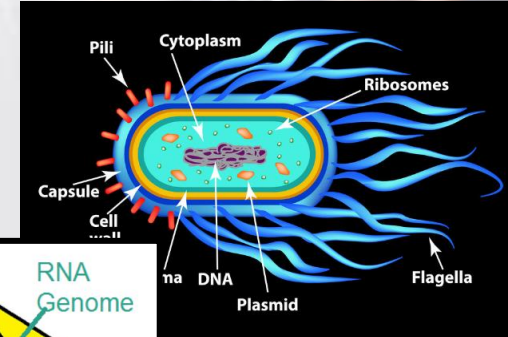


# Microbiological hazards

Numerous microbiological pathogens potentially linked to shellfish consumption

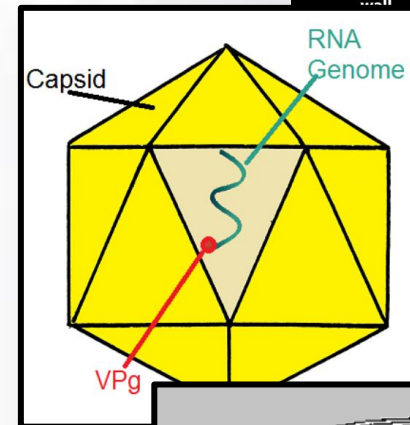
## BACTERIA

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



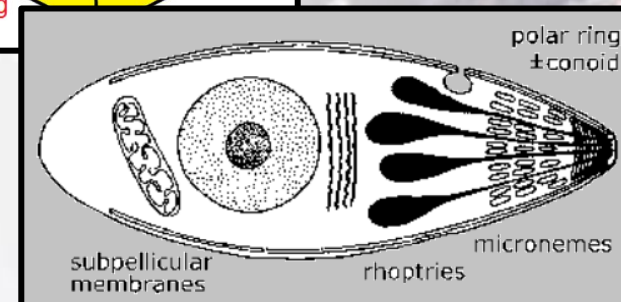
## VIRUSES

Norovirus, hepatitis A virus,  
sapovirus, hepatitis E virus



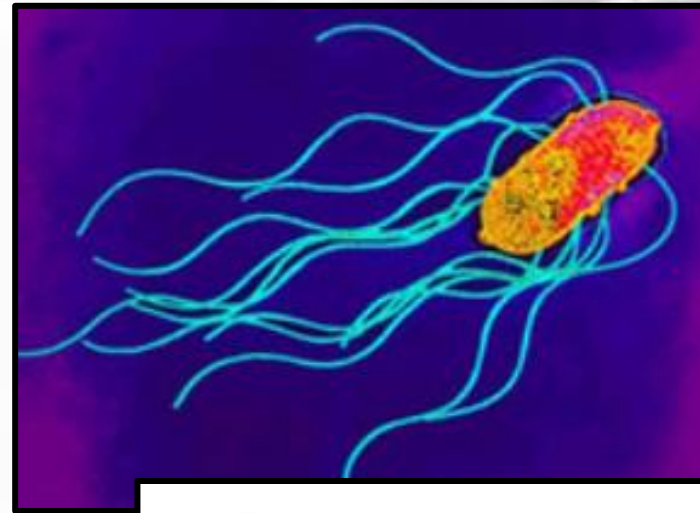
## PARASITES

*Giardia intestinalis*,  
*Cryptosporidium parvum*,  
*Microsporidia*



# *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

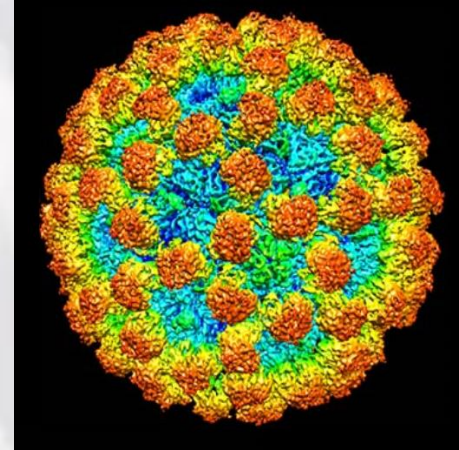
# *Vibrio parahaemolyticus* and *V. vulnificus*

- Gram negative bacterium
- Causes 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 **in some regions**

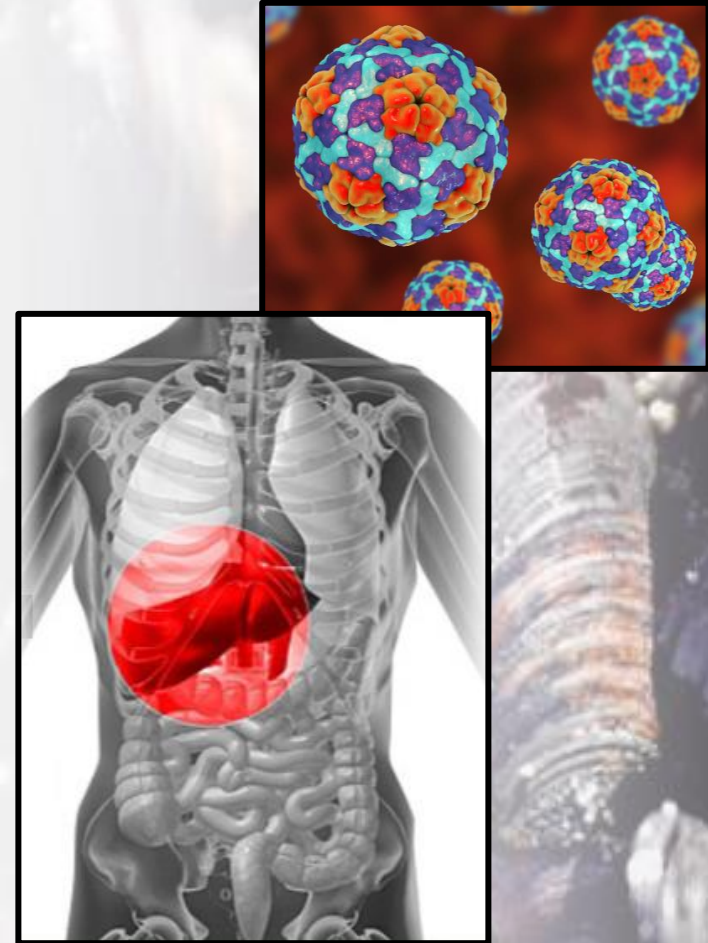


**NOROVIRUS:**  
YOU DON'T WANT IT.



# Hepatitis A virus

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





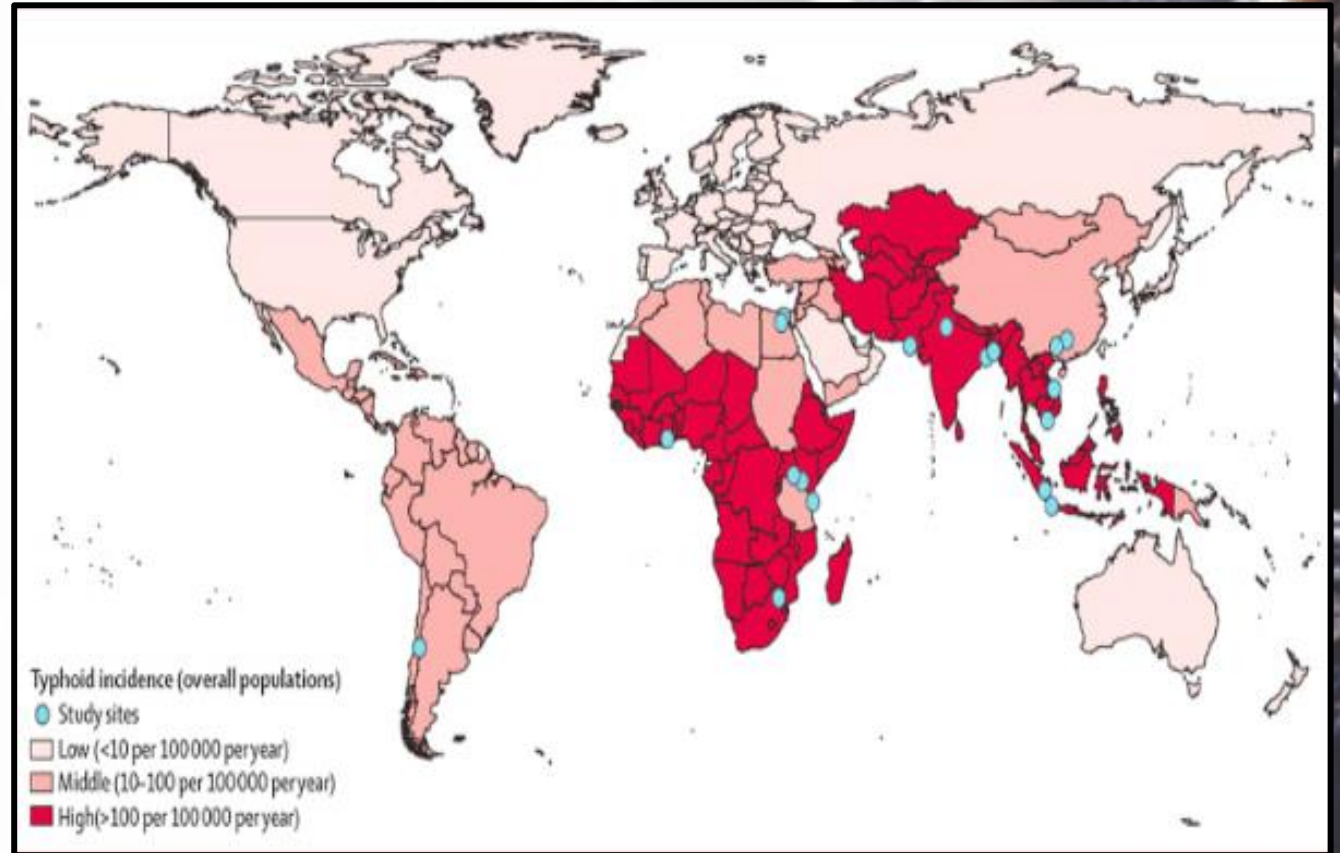
# Shellfish-related pathogens in Asia Pacific



- All major shellfish-related pathogens found in the Asian and Pacific general population
- Many reports of pathogen detection in shellfish samples
- Significant reports of shellfish-related outbreaks

# *Salmonella* spp.

High incidence of typhoid fever in parts of Asia Pacific



# *Salmonella* spp.

Salmonella regularly detected in surveys of shellfish in Asia Pacific – outbreaks have been reported occasionally

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

## Distribution and genotypic characterization of *Salmonella* serovars isolated from tropical seafood of Cochin, India

R. Kumar<sup>1</sup>, P.K. Surendran<sup>2</sup> and N. Thampuran<sup>1</sup>

<sup>1</sup> Microbiology, Fermentation and Biotechnology Division, Central Institute of Fisheries Technology, Cochin, India

<sup>2</sup> Poothuvalil, Dr. Surendran Lane, Perumpadappu, Cochin, India

Food Microbiology, 1995, 12, 3–8

ORIGINAL ARTICLE

## Prevalence of *Salmonella* in raw and cooked foods in Malaysia

R. K. Arumugaswamy,<sup>†</sup> G. Rusul,<sup>1\*</sup> S. N. Abdul Hamid<sup>1</sup> and C. T. Cheah<sup>2</sup>

> [Southeast Asian J Trop Med Public Health](#). 1981 Mar;12(1):55–62.

## An outbreak of paratyphoid A in Singapore: clinical and epidemiological studies

K T Goh

PMID: 6789457

### Abstract

The epidemiological and clinical findings of 61 cases of laboratory-confirmed paratyphoid A reported in an outbreak in 1979 were described. Epidemiological investigations of 42 indigenous cases implicated imported fresh oysters as the vehicles of transmission ( $p$  less than 0.01). Although *S. paratyphi* A could not be isolated from the implicated oysters, several observations presented tend to support the association between oyster consumption and illness. The clinical features were similar to typhoid, 82.8% of the organisms were isolated from blood cultures. The relapse rate was 8.6%. There were three stool and one urinary convalescent carriers. Two of the stool carriers were treated with cholecystectomy.

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 2007, p. 6885–6890

0099-2240/07/\$08.00+0 doi:10.1128/AEM.00972-07

Copyright © 2007, American Society for Microbiology. All Rights Reserved.

Vol. 73, No. 21

## Detection of *Salmonella* spp. in Retail Raw Food Samples from Vietnam and Characterization of Their Antibiotic Resistance<sup>∇</sup>

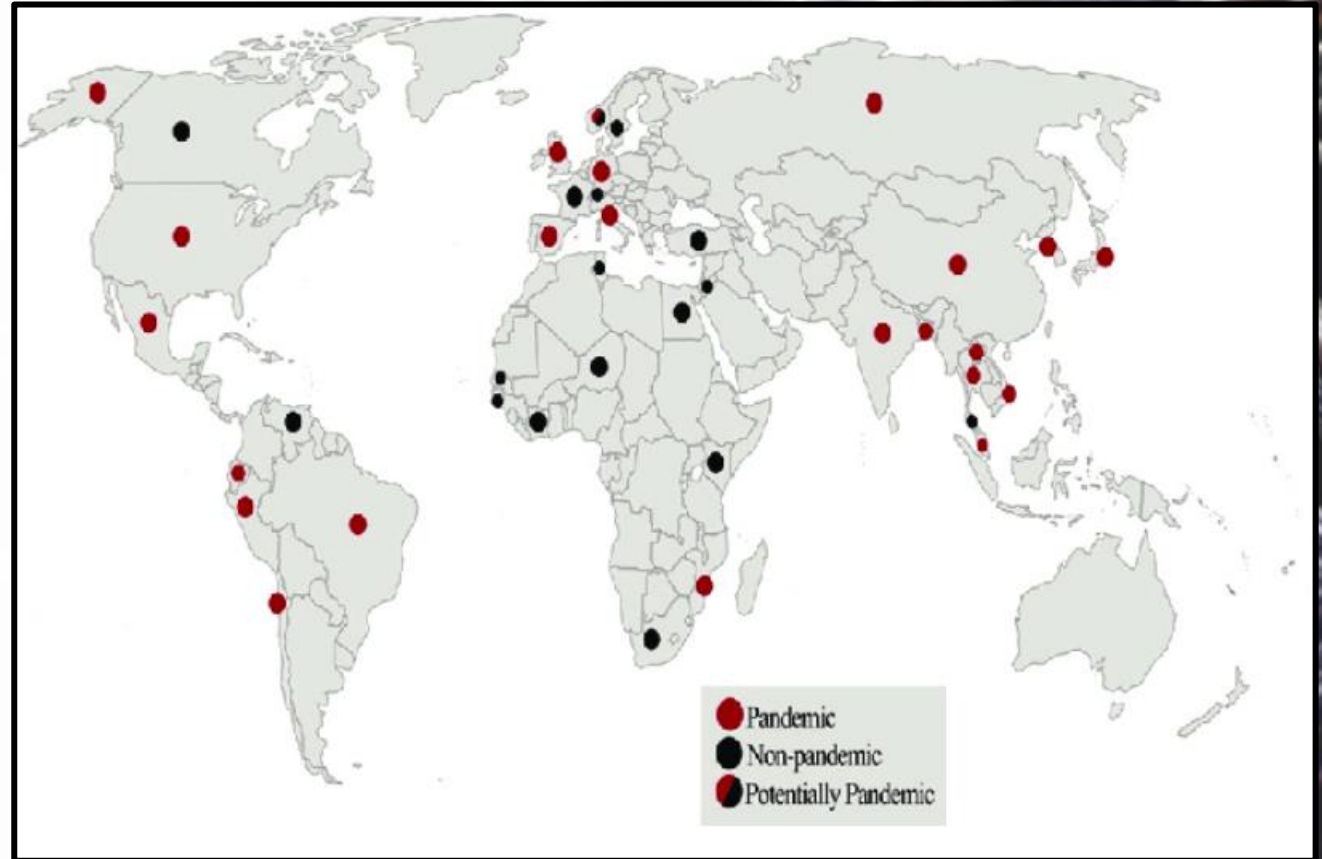
Thi Thu Hao Van,<sup>1</sup> George Moutafis,<sup>1</sup> Taghrif Istivan,<sup>1</sup> Linh Thuoc Tran,<sup>2</sup> and Peter J. Coloe<sup>1\*</sup>

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Received 30 April 2007/Accepted 19 August 2007

## *Vibrio spp.*

*Vibrio parahaemolyticus*  
recorded in many Asian  
countries (including  
pathogenic strains)



# *Vibrio* spp.

Many reports of detection of *Vibrio* spp. in shellfish and of shellfish-related strains in Asia Pacific

*Epidemiol. Infect.* (2004), 132, 993–996. © 2004 Cambridge University Press  
DOI: 10.1017/S0950268804002407 Printed in the United Kingdom

## SHORT REPORT

### *Vibrio vulnificus* septicaemia in Japan: an estimated number of infections and physicians' knowledge of the syndrome

K. OSAKA<sup>1</sup>\*, M. KOMATSUZAKI<sup>2,3</sup>, H. TAKAHASHI<sup>1</sup>, S. SAKANO<sup>4</sup>  
AND N. OKABE<sup>1</sup>

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(Accepted 12 March 2004)

JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3150–3155  
0095-1137/97/\$04.00+0  
Copyright © 1997, American Society for Microbiology

Vol. 35, No. 12

### 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

JUN OKUDA,<sup>1</sup> MASANORI ISHIBASHI,<sup>2</sup> ERIKO HAYAKAWA,<sup>3</sup> TAKESHI NISHINO,<sup>3</sup>  
YOSHIFUMI TAKEDA,<sup>4</sup> ASISH K. MUKHOPADHYAY,<sup>5</sup> SURABHI GARG,<sup>5</sup>  
S. K. BHATTACHARYA,<sup>5</sup> G. BALAKRISH NAIK,<sup>5</sup>  
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Center for Southeast Asian Studies, Kyoto University, Yoshida, Sakyo-ku, Kyoto,<sup>1</sup> Osaka Prefectural Institute of Public Health, Higashinari-ku, Osaka,<sup>2</sup> Department of Microbiology, Kyoto Pharmaceutical University, Yamashina, Kyoto,<sup>3</sup> and Research Institute, International Medical Center of Japan, Shinjuku-ku, Tokyo 162,<sup>4</sup> Japan, and Department of Microbiology, National Institute of Cholera and Enteric Diseases, Calcutta 700 010, India<sup>5</sup>

Received 14 July 1997/Returned for modification 28 August 1997/Accepted 24 September 1997

Tropical Medicine and Health Vol. 41 No. 3, 2013, pp. 95–102  
doi:10.2149/tmh.2011-06  
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## Original article

### Contamination by *Vibrio parahaemolyticus* and Its Virulent Strains in Seafood Marketed in Thailand, Vietnam, Malaysia, and Indonesia

Yoshitsugu Nakaguchi

Received 24 November, 2011 Accepted 19 April, 2012 Published online 19 June, 2013

## *Vibrio parahaemolyticus*, Southern Coastal Region of China, 2007–2012

Yinghui Li, Xu Xie, Xiaolu Shi, Yiman Lin, Yaqun Qiu, Jin Mou, Qiongcheng Chen, Yan Lu, Li Zhou, Min Jiang, Honghu Sun, Hanwu Ma, Jinquan Cheng, and Qinghua Hu

We analyzed the prevalence and characteristics of *Vibrio parahaemolyticus* among patients with acute infectious diarrhea in the southern coastal region of China. *V. parahaemolyticus* was the leading cause of bacterial infectious diarrhea in this region during 2007–2012. Serotype O3:K6 strains were most common, followed by serotypes O4:K8 and O3:K29.

# Norovirus

## Norovirus a common cause of gastroenteritis in Asia Pacific

Journal of Medical Virology

### Genetic Characterization of Norovirus Strains in Hospitalized Children From Pakistan

Amna Alam,<sup>1\*</sup> Sohail A. Qureshi,<sup>2</sup> Jan Vinje,<sup>3</sup> and Anita Zaidi<sup>1,4</sup>

<sup>1</sup>Department of Pediatrics and Child Health, The Aga Khan University Hospital, Karachi, Pakistan

<sup>2</sup>Department of Biology, Syed Babar Ali School of Science and Engineering, Lahore University of Management Sciences, Lahore, Pakistan

<sup>3</sup>Division of Viral Diseases, Centers for Disease Control and Prevention (CDC), Atlanta, Georgia

<sup>4</sup>Enteric and Diarrheal Diseases, Global Health, Bill and Melinda Gates Foundation, North Seattle, Washington

Hindawi Publishing Corporation  
Journal of Tropical Medicine  
Volume 2016, Article ID 2707121, 8 pages  
<http://dx.doi.org/10.1155/2016/2707121>

Research Article

### Molecular Epidemiology and Genetic Diversity of Norovirus in Young Children in Phnom Penh, Cambodia

Kaewkanya Nakjarung,<sup>1</sup> Ladaporn Bodhidatta,<sup>1</sup> Pimnapar Neesanant,<sup>1</sup>  
Paphavee Lertsethtakarn,<sup>1</sup> Orntipa Sethabutr,<sup>1</sup> Ket Vansith,<sup>2</sup>  
Chhour Y. Meng,<sup>2</sup> Brett E. Swierczewski,<sup>1</sup> and Carl J. Mason<sup>1</sup>

<sup>1</sup>Department of Enteric Diseases, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Road, Bangkok 10400, Thailand

<sup>2</sup>National Pediatric Hospital, 100 Russian Federation Boulevard, Phnom Penh, Cambodia

Correspondence should be addressed to Ladaporn Bodhidatta; [ladapornb@afirms.org](mailto:ladapornb@afirms.org)

### Norovirus GII.21 in Children with Diarrhea, Bhutan

Takaaki Yahiro, Sonam Wangchuk, Takeshi Wada, Chimmi Dorji, Takashi Matsumoto, Mimi Lhamo Mynak, Kunzang Pem Tshering, Akira Nishizono, Kamruddin Ahmed

Author affiliations: Oita University, Yufu, Oita, Japan (T. Yahiro, T. Wada, T. Matsumoto, A. Nishizono, K. Ahmed); Ministry of Health, Royal Government of Bhutan, Thimphu, Bhutan (S. Wangchuk, C. Dorji); Jigme Dorji Wangchuk National Referral Hospital, Thimphu (M.L. Mynak); University of Medical Sciences, Royal Government of Bhutan, Thimphu (K.P. Tshering)


DOI: <http://dx.doi.org/10.3201/eid2104.141856>

Archives of Virology (2019) 164:1515–1525  
<https://doi.org/10.1007/s00705-019-04215-y>

ORIGINAL ARTICLE



### Norovirus and rotavirus infections in children less than five years of age hospitalized with acute gastroenteritis in Indonesia

Hera Nirwati<sup>1</sup> · Celeste M. Donato<sup>2,3</sup> · Yuli Mawarti<sup>4</sup> · Nenny S. Mulyani<sup>5</sup> · Aqsa Ikram<sup>6,7</sup> · Abu T. Aman<sup>1</sup> · Maikel P. Peppelenbosch<sup>6</sup> · Yati Soenarto<sup>5</sup> · Qiuwei Pan<sup>6</sup> · Mohamad S. Hakim<sup>1,6</sup> 

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# Norovirus

Norovirus regularly detected in surveys of shellfish in Asia Pacific – outbreaks have been reported

## NOROVIRUSES IN OYSTERS FROM LOCAL MARKETS AND OYSTER FARMS IN SOUTHERN THAILAND

Leera Kittigul, Kannika Pombubpa, Suntharee Sukonthalux, Tippawan Rattanatham and Fuangfa Utrarachkij

Department of Microbiology, Faculty of Public Health, Mahidol University, Bangkok, Thailand

*Epidemiol. Infect.* (2016), **144**, 2759–2764. © Cambridge University Press 2016  
doi:10.1017/S0950268816000170

## An outbreak of norovirus infection associated with fermented oyster consumption in South Korea, 2013

H. G. CHO<sup>1</sup>, S. G. LEE<sup>2</sup>, M. Y. LEE<sup>3</sup>, E. S. HUR<sup>1</sup>, J. S. LEE<sup>4</sup>, P. H. PARK<sup>1</sup>, Y. B. PARK<sup>1</sup>, M. H. YOON<sup>1</sup> AND S. Y. PAIK<sup>5\*</sup>

<sup>1</sup> Division of Public Health Research, Gyeonggi Province Institute of Health and Environment, Suwon, Republic of Korea

<sup>2</sup> Korea Zoonosis Research Institute, Chonbuk National University, Iksan, Republic of Korea

<sup>3</sup> An-Seong Public Health center, An-Seong, Republic of Korea

<sup>4</sup> Food Microbiology Division, Food Safety Evaluation Department, National Institute of Food and Drug Safety Evaluation, Osong, Republic of Korea

<sup>5</sup> Department of Microbiology, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Received 31 August 2015; Final revision 16 November 2015; Accepted 14 January 2016;  
first published online 2 February 2016

► *Food Environ Virol.* 2018 Mar;10(1):61-71. doi: 10.1007/s12560-017-9325-1. Epub 2017 Nov 7.

## Oyster Contamination with Human Noroviruses Impacted by Urban Drainage and Seasonal Flooding in Vietnam

Gia Thanh Nguyen<sup>1 2 3</sup>, Jian Pu<sup>4</sup>, Takayuki Miura<sup>5</sup>, Hiroaki Ito<sup>6</sup>, Shinobu Kazama<sup>7</sup>, Yoshimitsu Konta<sup>8</sup>, An Van Le<sup>9</sup>, Toru Watanabe<sup>10</sup>

PMID: 29230695 DOI: 10.1007/s12560-017-9325-1

*European Journal of Epidemiology* 15: 175–180, 1999.  
© 1999 Kluwer Academic Publishers. Printed in the Netherlands.

## Outbreaks of gastroenteritis caused by SRSVs from 1987 to 1992 in Kyushu, Japan: Four outbreaks associated with oyster consumption

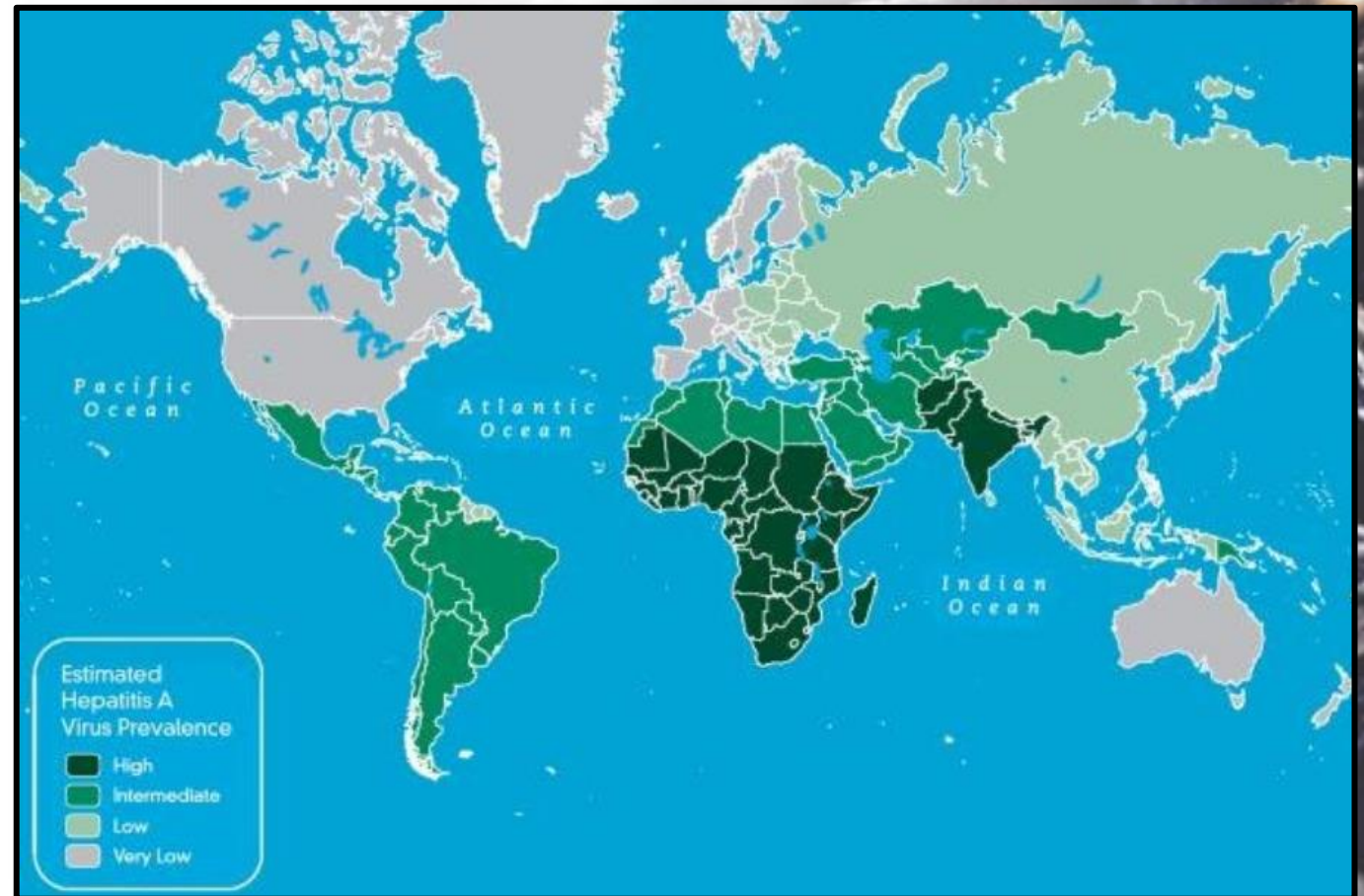
Ryuichi Otsu

Fukuoka Institute of Health and Environmental Sciences, Mukaizano, Dazaifu, Fukuoka, Japan

Accepted in revised form 23 October 1998

# Hepatitis A virus

High/intermediate prevalence of HAV in some parts of Asia Pacific





# Hepatitis A virus

HAV regularly detected in surveys of shellfish in Asia Pacific  
– outbreaks have been reported

Letters in Applied Microbiology 

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

**Surveillance of hepatitis A and E viruses contamination in shellfish in Thailand**

A. Namsai<sup>1</sup>, S. Louisirirothanakul<sup>1</sup>, N. Wongchinda<sup>2</sup>, U. Siripanyaphinyo<sup>3</sup>, P. Virulhakul<sup>4</sup>, P. Puthavathana<sup>1</sup>, K.S. Myint<sup>5</sup>, M. Gannarong<sup>6</sup> and R. Ittapong<sup>2</sup>

<sup>1</sup> Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand  
<sup>2</sup> Fishery Technological Development Division, Bangkok, Thailand  
<sup>3</sup> Research Collaboration Center on Emerging and Re-emerging Infections (RCC-ERI), Department of Medical Sciences, Nonthaburi, Thailand  
<sup>4</sup> Central Government, Department of Fisheries, Bangkok, Thailand  
<sup>5</sup> Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand  
<sup>6</sup> Fish Inspection and Quality Control Division, Surat Thani, Thailand

Bulletin of the World Health Organization, 62 (6): 893 – 897 (1984) © World Health Organization 1984

**An epidemic of cockles-associated hepatitis A in Singapore**

K. T. GOH,<sup>1</sup> L. CHAN,<sup>2</sup> J. L. DING,<sup>2</sup> & C. J. OON<sup>3</sup>

*An epidemic of serologically confirmed hepatitis A occurred between May and September 1983 in Singapore. The vehicle of transmission was traced to raw and partially cooked cockles, Anadara granosa, which had been imported from places with no sanitary control on the production. Strict controls on imported cockles are warranted.*

Epidemiol. Infect. (2000), 124, 121–130. Printed in the United Kingdom © 2000 Cambridge University Press

**Hepatitis A in New South Wales, Australia, from consumption of oysters: the first reported outbreak**

S. CONATY<sup>1</sup>, P. BIRD<sup>2</sup>, G. BELL<sup>3</sup>, E. KRAA<sup>4</sup>, G. GROHMANN<sup>5</sup>  
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<sup>1</sup> AIDS/Infectious Diseases Branch, NSW Health Department, Locked Mail Bag 961, North Sydney, NSW Australia 2059  
<sup>2</sup> Hunter Public Health Unit, NSW, Australia  
<sup>3</sup> Northern Districts Public Health Unit, NSW, Australia  
<sup>4</sup> Food and Nutrition Branch, NSW Health Department, NSW, Australia  
<sup>5</sup> Department of Veterinary Anatomy and Pathology, University of Sydney, Australia

(Accepted 6 September 1999)

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**An Epidemic of Hepatitis A Attributable to the Ingestion of Raw Clams in Shanghai, China**

Mabel L. Halliday, Lai-Yi Kang, Ting-Kui Zhou, Meng-Dong Hu, Qi-Chao Pan, Ting-Yuan Fu, Yu-Sheng Huang, and Shan-Lian Hu

Department of Preventive Medicine and Biostatistics, Faculty of Medicine, University of Toronto, Canada; Shanghai Hygiene and Anti-Epidemic Center, Nan-Shi and Hong-Kou Hygiene and Anti-Epidemic Stations, and Shanghai Medical University, People's Republic of China

# Hepatitis A virus

Largest ever outbreak of shellfish-related illness due to HAV in clams in Shanghai, China; almost 300,000 people affected

852

## **An Epidemic of Hepatitis A Attributable to the Ingestion of Raw Clams in Shanghai, China**

**Mabel L. Halliday, Lai-Yi Kang, Ting-Kui Zhou,  
Meng-Dong Hu, Qi-Chao Pan, Ting-Yuan Fu,  
Yu-Sheng Huang, and Shan-Lian Hu**

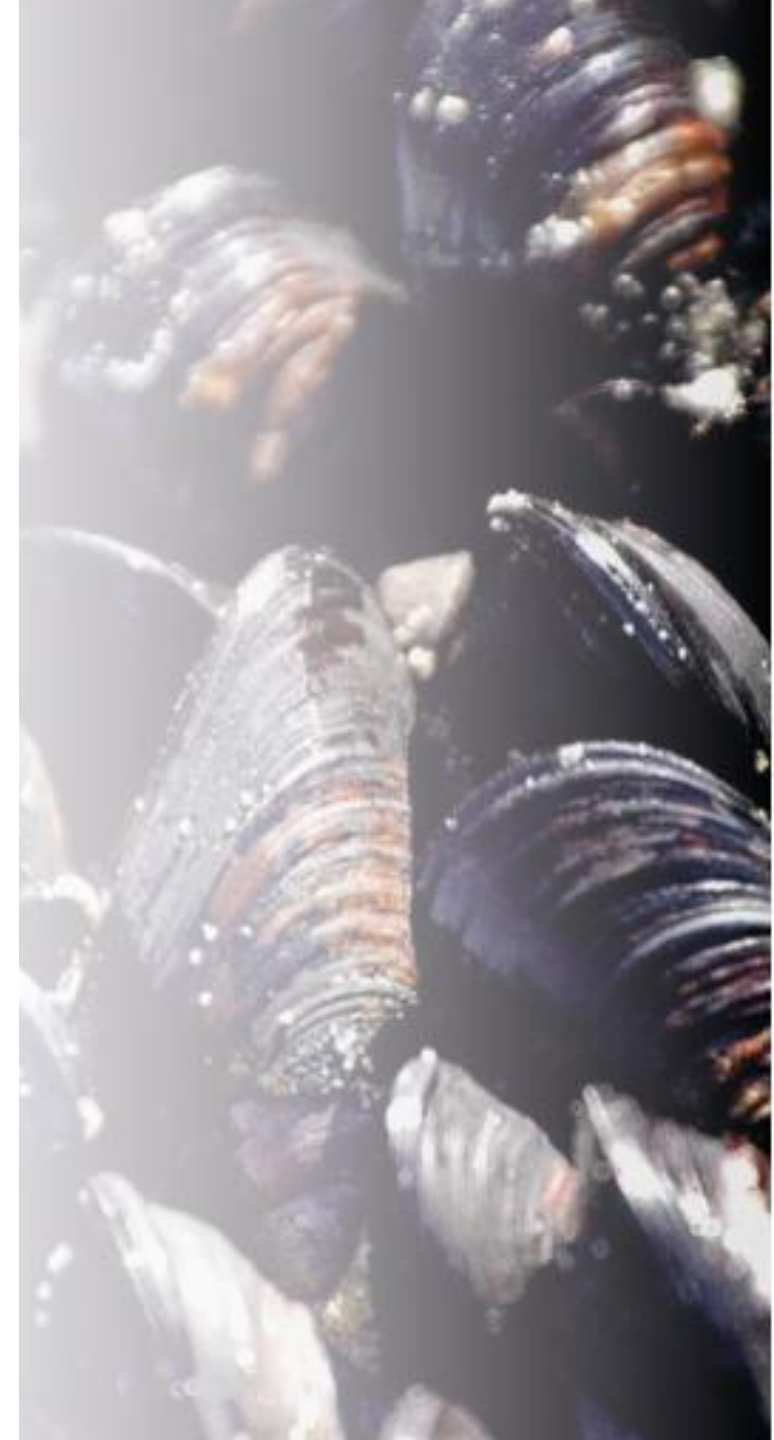
*Department of Preventive Medicine and Biostatistics, Faculty of Medicine, University of Toronto, Canada; Shanghai Hygiene and Anti-Epidemic Center, Nan-Shi and Hong-Kou Hygiene and Anti-Epidemic Stations, and Shanghai Medical University, People's Republic of China*

An epidemic of hepatitis A in 1988 in Shanghai had an overall attack rate of 4083/100,000 population (292,301 cases). The epidemic curve showed three peaks in January and February. A case-control study of 1208 matched pairs supported that clams were the vehicle for the virus (summary odds ratio, 9.47;  $P < .001$ ). Analysis of subsets who had eaten clams indicated that only 3.5% with hepatitis A had cooked their clams compared with 18.1% without hepatitis A, and those with the disease consumed more clams. A historical cohort study indicated that ~31.7% of the population had eaten clams one or more times between 9 December 1987 and 3 January 1988. The estimated attack rates in those who had and had not eaten clams were 11.93% and 0.52%, respectively (relative risk, 22.94; attributable risk, 11.41%). The three peaks in the consumption curve correlated with those in the epidemic curve. Hepatitis A virus was demonstrated in clams taken from the Shanghai markets and from the catching area.

# 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 –  
**NOTE absence of data does not necessarily imply absence of risk**

**PRINCIPAL SOURCE AND FACTOR CONSIDERATIONS FOR THE MAJOR HAZARD GROUPS**



# 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) samples 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
- Risks posed by different hazards may depend on the characteristics of the growing area
- Specific testing for hazards may require specialist laboratories

