



# Human Pathogenic Viruses in Bivalve Molluscan Shellfish



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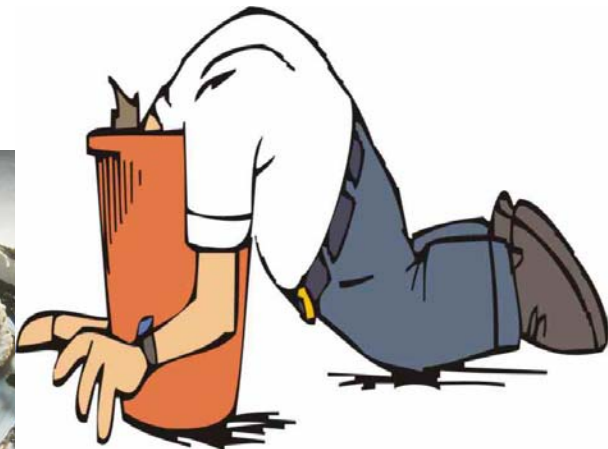
# Presentation Overview

- **Public health problems associated with molluscan bivalve shellfish**
  - Sewage contamination
- **Viral illness**
  - Norovirus
  - Hepatitis
- **Progress in controlling viral risks**
  - Method development
  - Risk based management approach
  - SEAFOODplus



# Shellfish and Public Health

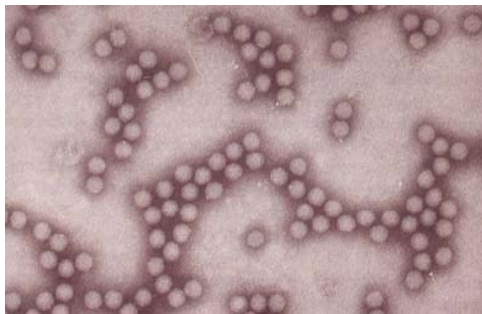
- Many shellfish areas are also impacted by sewage
- Shellfish feed by filtration
- Sewage-derived pathogens are concentrated
- Shellfish are eaten raw or lightly cooked
- Outbreaks of disease can occur





# Viral illness in shellfish

- The largest recorded outbreak of food-borne virus disease in history
  - *China, March 1988*
  - *Clams from Shanghai caused more than 292,000 cases of hepatitis A*
  - *9 Deaths*





# Public health controls



Control of sewage pollution  
(Shellfish Waters Directive 79/923/EEC)



Classification and monitoring  
of harvesting areas



Commercial processing  
(depuration, relaying, cooking)



End-product controls  
(quality tests, traceability)

All use *E. coli* (bacteria) monitoring for control



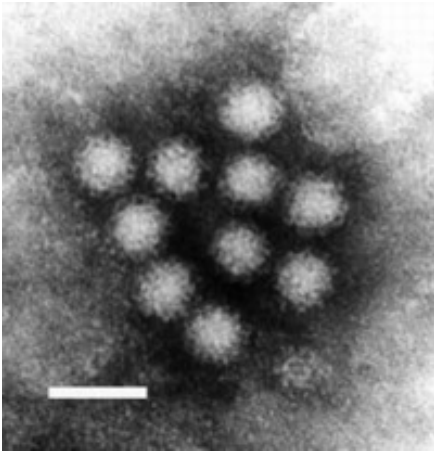
# Published outbreaks (illustrative)

- **Scandinavia, January 1997**
  - *Gastro-enteritis associated with oysters produced in Europe*
  - *Denmark 356 documented cases, also Sweden, Holland and Germany*
  - *approx 35,000 contaminated oyster sold in Denmark alone*
- **Spain, September 1999**
  - *Hepatitis A associated with frozen clams imported from Peru*
  - *183 confirmed cases*
- **France and Italy, December 2002**
  - *Norovirus gastro-enteritis associated with oysters produced in Etang de Thau, France*
  - *France 14 outbreak clusters, 69 confirmed cases; Italy 200+ cases*



# Pathogenic viruses in Shellfish

- **Norovirus (SRSVs, NLVs)**



- ‘mild’ gastroenteritis, including nausea, diarrhoea, vomiting, fever and abdominal pain
- IP 1-4 days, duration of about 2 days
- The most common cause of IID disease
- Many genetically diverse strains of norovirus

- **Hepatitis A Virus –Southern Europe**

- Extended IP of about 4 weeks (range 2 to 6)
- Serious debilitating disease with fever, headache, nausea, vomiting, diarrhoea, abdominal pain and jaundice
- Self-limiting and rarely causes death but patients may be incapacitated for several months



# Controlling viral risks -The Way Forward?

- **Method standardisation and validation**
- **Risk based management procedures**
- **SEAFOODplus integrated Research Project**
  - *Development of standard reference methods for hepatitis A and norovirus in bivalve molluscan shellfish (REFHEPA)*
    - *Albert Bosch (University of Barcelona)*
  - *Reduction of Risk in shellfish harvesting areas (REDRISK)*
    - *Monique Pommepuy (IFREMER)*



# Pathogenic viruses are difficult to detect

- **NV and HAV detection in shellfish is difficult**
  - *Not easily Cultured (impossible for NVs)*
  - *Low levels compared with clinical samples*
  - *Shellfish are very inhibitory in tests*
  - *High level of strain diversity (norovirus)*
- **Molecular methods offer only route for detection and PCR methods have been developed**
  - *Real-time quantitative methods*



## Comparison of Conventional PCR and Real Time PCR

	<b>Conventional RT-PCR</b>	<b>Real Time PCR (TaqMan)</b>
<b>Quantitation</b>	<b>No</b>	<b>Yes</b>
<b>Detection</b>	<b><i>Gel electrophoresis</i></b>	<b><i>One step detection</i></b>
<b>Confirmation</b>	<b><i>Sequencing</i></b>	<b><i>In built probe confirmation</i></b>
<b>Sensitivity</b>	<b><i>Nested PCR (contamination)</i></b>	<b><i>Flourescent probe technology</i></b>
<b>Infectivity</b>	<b><i>Unknown</i></b>	<b><i>Unknown</i></b>



# REFHEPA PROJECT

## Objective;

To develop and validate quantitative methods for the detection of **HAV and NV** in shellfish to **International Standard Organisation (ISO)** standards.

- **48 months starting Jan 2004**
- **6 European partners**
  - *Develop and validate assays*
  - *Produce control reagents*
  - *Interlaboratory collaboration (NRLs)*
  - *Standardised real-time PCR methods*

*(CEN working group on horizontal virus method for food)*



# REDRISK Objectives

- To identify pollution sources and conditions responsible for **viral contamination** in shellfish. To provide a framework for a risk management approach to controlling the the viral risk associated with shellfish during primary production.
  - *Identify pollution sources in harvesting areas.*
  - *Determine impact of pollution sources and environmental conditions on virus contamination.*
  - *Develop risk management strategy (future)*



# REDRISK APPROACH

- **Select commercial harvesting areas in 4 European countries**
  - UK, France, Spain & Ireland
  - Good quality Cat B sites showing intermittent contamination
- **Identify microbial pollution sources in harvesting areas.**
  - Sewage treatment works
  - Septic tanks etc.
  - Non-point pollution sources (animal)
- **Determine impact of pollution sources and environmental conditions on **virus contamination**.**
  - Rainfall, wind, tides
  - Virus levels, levels of indicator organisms, link with illness
- **Develop risk management strategy (after 36 months)**
  - Restrict harvesting
  - Control measures (depuration/relaying)



# SUMMARY

- **Real-Time quantitative PCR methods for detection of viruses in shellfish have been developed**
- **Efforts at validation & standardisation are well advanced**
  - REFHEPA, CEN Working group, NRLs network
- **Virus methods are assisting in the development of risk based management programmes for shellfisheries (REDRISK)**
- **Development of both standardised methods and management programmes will provide tools for producing safer shellfish in Europe**