

I principali pericoli sanitari legati al consumo dei prodotti ittici


LEZIONE N. 4

Giuseppe Arcangeli
Ferrara – 29 ottobre 2009



Live bivalve molluscs

LBM: filtering organisms

	°C	Lt/h
Mussel	14	1,5
European Oyster (<i>O. edulis</i>)	15	12
American Oyster (<i>C. virginica</i>)	20	18

A lot of biological particles (bacteria, viruses, biotoxins, chemical compounds) accumulate into LBM



Mollusc production in Italy (tons) in 2007



100.000 t (40 %)



50.000 t (80 %)



30.000 t (30-40 %)



Other species:

Donax trunculus
(Donax clams)



Ensis spp.
(Razor clam)



Crassostrea gigas
(Pacific oyster)



Clamys spp.
(Queen scallop)



Callista chione
(Hard clam)



Paracentrotus lividus
(Sea urchin)



Murex spp.
(Murex)



Nassarius mutabilis
(Mutable nassa)



Dir. 60/2000
Dir. 113/2006

The diagram consists of two overlapping circles on a green background. The left circle is outlined in red and is associated with the Ministry of Environment. The right circle is outlined in yellow and is associated with the Ministry of Health. The intersection of the two circles is labeled 'Safety LBM' in red text. The text 'Dir. 60/2000' and 'Dir. 113/2006' is located in the red-outlined circle. The text 'Reg. 853/2004', 'Reg. 854/2004', and 'Reg. 2073/2005' is located in the yellow-outlined circle. The text 'Ministry of Environment' is at the bottom of the red circle, and 'Ministry of Health' is at the bottom of the yellow circle.

Ministry of Environment

Safety LBM

Reg. 853/2004
Reg. 854/2004
Reg. 2073/2005

Ministry of Health

Bivalves Molluscs safety

(Ministry of Health)

- ✓ Monitoring on **microbiological** parameters, **biotoxins** and **toxic phytoplankton** in bivalves harvesting areas
- ✓ Chemicals control: **heavy metals**



Bivalves Molluscs Safety

Ministry of Environment

- ✓ Physical parameters (pH, Oxig., T, Turb., Sal.)
- ✓ Microbiological (Faecal coliforms)
- ✓ Chemicals control: heavy metals (Pb, Cd, Hg)

PCB, IPA

TABELLA 1 (NOTIFICA DI ALLERTA)

DATA	NOTIFICATO:	REF:	PRODOTTO	MOTIVAZIONE	ORIGINE
12/02/07	REP. CECA	2007.0109	Salmone della Norvegia affumicato	Listeria monocytogenes	POLONIA
14/02/07	REP. CECA	2007.0112	Mangime per pesci	Frammenti di lische	PERU→GERMANIA
14/02/07	ITALIA	2007.0113	Vongola (Venus verrucosa) →	E. coli, Salmonella spp	GRECIA
15/02/07	ITALIA	2007.0117	Cozza (Mytilus galloprovincialis) →	Escherichia coli	SLOVENIA
16/02/07	REP. CECA	2007.0119	Salmone della Norvegia affumicato	Listeria monocytogenes	POLONIA
16/02/07	ITALIA	2007.0122	Scampo(Nephrops norvegicus)	Solfiti	U.K.
16/02/07	ITALIA	2007.0124	Scampo(Nephrops norvegicus)	Solfiti	U.K.
20/02/07	SPAGNA	2007.0129	Tonno e pesce spada (carpaccio)	Monossido di carbonio	VIETNAM→OLANDA
21/02/07	DANIMARCA	2007.0136	Cozza cilena sgusciata (Mytilus chilensis) →	Escherichia coli	CILE
21/02/07	NORVEGIA	2007.0137	Tonno	Monossido di carbonio	INDONESIA→OLAND
21/02/07	R. SLOVACCA	2007.0138	Salmone della Norvegia affumicato	Listeria monocytogenes	POLANIA
21/02/07	ITALIA	2007.0140	Pesce spada (Xiphia gladius)	Mercurio	SPAGNA
22/02/07	R. SLOVACCA	2007.0142	Salmone della Norvegia affumicato	Listeria monocytogenes	REP. CECA
22/02/07	ITALIA	2007.0145	Pesce spada sotto vuoto	mercury	DANIMARCA
22/02/07	ITALIA	2007.0148	Scampo(Nephrops norvegicus)	Solfiti	U.K.
27/02/07	SPAGNA	2007.0156	toxins in frozen scallops	DSP	FRANCIA
27/02/07	ITALIA	2007.0159	Pesce spada	Mercurio	SPAGNA
28/02/07	ITALIA	2007.0162	Cozza (Mytilus galloprovincialis) →	Escherichia coli	SLOVENIA
28/02/07	FRANCIA	2007.0163	Vongola verace (Tapes semidecussatus) →	E. coli, Salmonella spp	ITALIA
01/03/07	ITALIA	2007.0166	Scampo(Nephrops norvegicus)	Solfiti	U.K.

- Election Test ISO TS 16649-3
(Donovan method: attività β -glucuronidase di E.coli)
- Accredited laboratory according EN ISO /IEC 17025



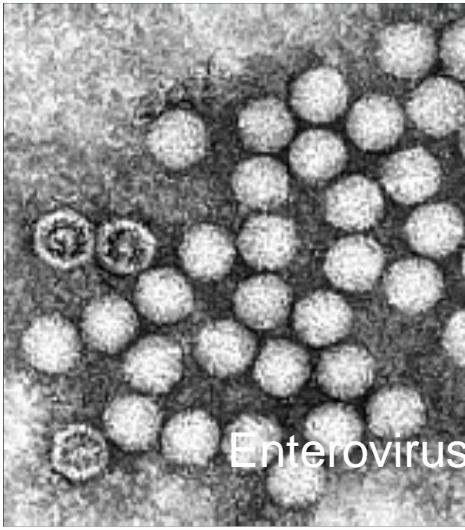
Emerging pathogens transmissible by mollusc to consumers

Viruses: *HAV, Norovirus, Enterovirus (Coxsackievirus, etc.)*

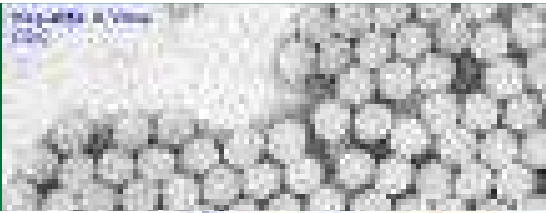
Bacteria: *Vibrio parahaemolyticus, Vibrio vulnificus, NAG vibrios*

Protozoa: *Giardia, Toxoplasma, Cryptosporidium*

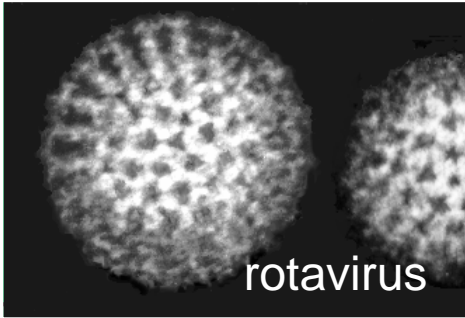




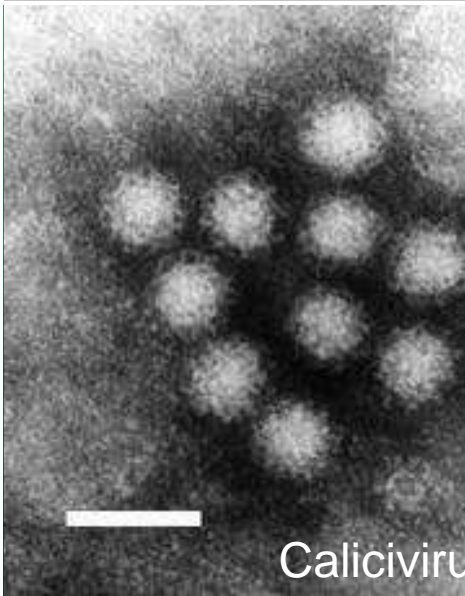
Enterovirus



Mycobacterium



rotavirus



Calicivirus



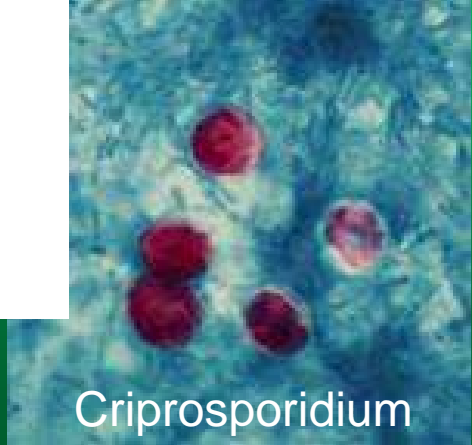
Vibrio



Toxoplasma



Giardia



Cripsporidium

Vibrio vulnificus e **Vibrio parahaemolyticus**: autoctonous in seafood

Reg. 2073/2005 ,initial considerations.....

(11) The SCVPH adopted an opinion on *Vibrio vulnificus* and *Vibrio parahaemolyticus* on 19 and 20 September 2001. It concluded that currently available scientific data do not support setting specific criteria for pathogenic *V. vulnificus* and *parahaemolyticus* in seafood. However, it recommended that codes of practice should be established to ensure that good hygiene practice has been applied.

(27) In particular, criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. There is a need for development of reliable methods for other microbial hazards too, e.g. *Vibrio parahaemolyticus*.

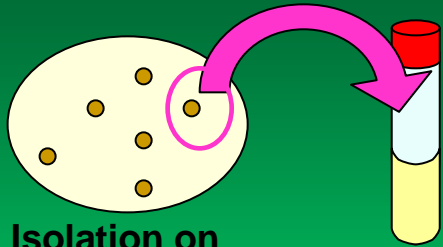
Only **TDH** and **TRH** positive are dangerous

When ingested, *V. parahaemolyticus* causes watery diarrhea often with abdominal cramping, nausea, vomiting fever and chills.

Usually these symptoms occur within 24 hours of ingestion. Illness is usually self-limited and lasts 3 days.

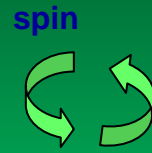
Severe disease is rare and occurs more commonly in persons with weakened immune systems.

PCR - *V. parahaemolyticus*

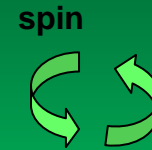
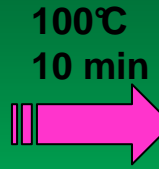


Isolation on
TSA 2%NaCl

TSB 3%NaCl
30°C O.N.



+ H₂O

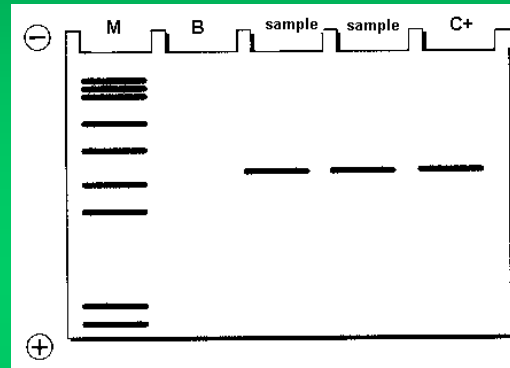


surnatant

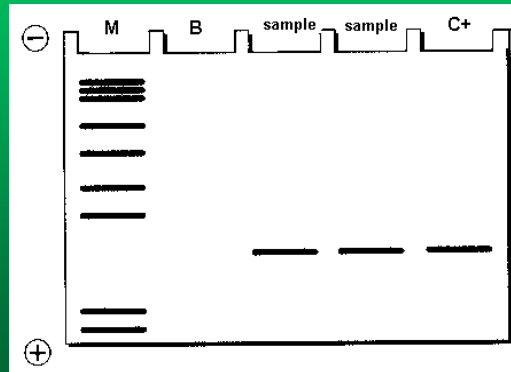
***Vibrio
parahaemolyticus***

C⁺
ATCC 43996 → TDH
ATCC 17802 → TRH

***Vibrio
parahaemolyticus*
toxins TRH / TDH
producer**



PCR
toxR



PCR
TRH / TDH

Reg. 2073/2005 ,initial consideration.....

(27) In particular, criteria for pathogenic viruses in live bivalve molluscs should be established when the analytical methods are developed sufficiently. There is a need for development of reliable methods for other microbial hazards too, e.g. *Vibrio parahaemolyticus*.

WHAT ARE THE SYMPTOMS?

Symptoms usually occur within 24-48 hours, and may include:

- Fever / Chills
- Skin lesions
- Stomach pain / Nausea
- Vomiting
- Diarrhea
- Shock

If you have consumed raw shellfish, and have any of these symptoms, **seek medical attention immediately.**

For those at risk, infection can lead to death within two days. Early, aggressive antibiotic treatment is the most effective therapy.

Vibrio vulnificus rarely affects healthy individuals. When it does, symptoms are mild and temporary.

FOR MORE INFORMATION

Contact the following:

- FDA Food Safety hotline: **1-888-723-3366**
- FDA website: www.cfsan.fda.gov
- ISSC website: www.issc.org

COOKING TIPS IN THE SHELL

- Cook live oysters or clams in small pots so those in the middle are cooked thoroughly.
- **BOILING:** After the shells open, boil live oysters or clams for another 3-5 minutes.
- **STEAMING:** In a pot that is already steaming, cook live oysters or clams for another 4-9 minutes.

SHUCKED OYSTERS

- **BOIL** or **SIMMER** for at least 3 minutes or until edges curl
- **FRY** at 375°F for at least 3 minutes
- **BROIL** 3 inches from heat for 3 minutes
- **BAKE** at 450°F for 10 minutes



INTERSTATE SHELLFISH
SANITATION CONFERENCE
(803) 788-7559 • www.issc.org

The American Liver Foundation has reviewed the contents of this brochure and supports the efforts of the ISSC in educating at-risk consumers. For information on liver disease and hepatitis, contact:



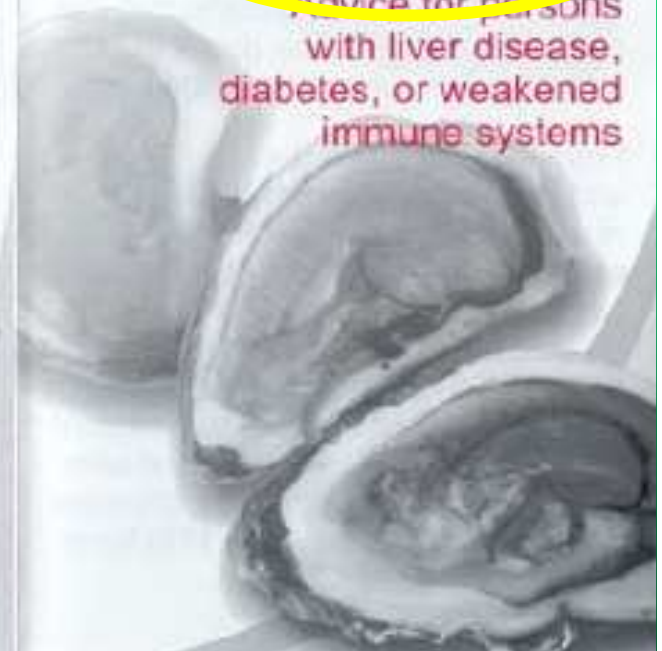
1-800-GO-LIVER (465-4837)
www.liverfoundation.org

This information is intended to provide general information only. This information does not constitute medical advice, and is not intended to be used as a substitute for professional medical advice. The American Liver Foundation (ALF) does not endorse any products or services. If you have any questions or concerns, please contact your physician or other healthcare provider. We do not recommend that you cancel any planned liver surgery or any course of treatment.

The Risk of Eating Raw Molluscan Shellfish

Containing *Vibrio vulnificus*

Advice for persons with liver disease, diabetes, or weakened immune systems



DID YOU KNOW...?

Each year millions of Americans enjoy eating raw molluscan shellfish--especially oysters and clams. But if you have **liver disease, diabetes,** or a **weak immune system,** raw shellfish containing the bacteria *Vibrio vulnificus* can make you seriously ill.

You can avoid illness simply by abstaining from consumption of raw shellfish. Eat only shellfish that have been thoroughly cooked.

WHAT IS VIBRIO VULNIFICUS?

Vibrio vulnificus is a bacteria that can cause severe illness or death to at-risk people who eat raw shellfish.

From 1989 to 2002, the U.S. Food and Drug Administration (FDA) recorded 341 serious illnesses associated with consumption of raw oysters and clams containing the *Vibrio vulnificus* bacteria. Ninety-eight percent (98%) of these illnesses have been associated with consumption of raw oysters with 2% associated with Eastern Hard Clams. While illnesses are infrequent, about half (179) have resulted in death.

WHERE IS IT FOUND?

Vibrio vulnificus is found naturally in warm coastal waters, such as the Gulf of Mexico, where levels of the bacteria are elevated during the summer months. *Vibrio vulnificus* is **NOT** a result of pollution, and can be found in waters approved for shellfish harvesting. *Vibrio vulnificus* does **NOT** change the appearance, taste, or odor of shellfish.

ARE YOU AT RISK?

You are at risk of serious illness if you eat raw shellfish and have any of these health conditions:

- **Liver disease** (from hepatitis, cirrhosis, alcoholism, or cancer)
- **Iron overload disease** (hemochromatosis)
- **Diabetes**
- **Cancer** (including lymphoma, leukemia, Hodgkin's disease)
- **Stomach disorders**
- **Or any illness or medical treatment that weakens the body's immune system**

Unsure of your risk? Ask your doctor.

Healthy people are not at risk of serious infection.

HOW CAN YOU AVOID INFECTION?

If you are at risk, raw or undercooked shellfish containing *Vibrio vulnificus* can make you sick.

You can also become infected if these bacteria enter your body through an open wound while swimming.

To safeguard your health, take these precautions:

- Physicians recommend that those at risk not eat any food of raw animal protein origin. This includes raw shellfish.
- **EAT** oysters or clams that have been **THOROUGHLY COOKED**--heat destroys the bacteria
- **NEVER** swim or wade in seawater when you have sores or open wounds



July 2004: outbreak in Alaska
after raw oysters consumption

$T_{\text{water}} > 15\text{ }^{\circ}\text{C}$

(Mc Laughlin et al., N. Engl. J. Med. 2005)

60 °N





Some enteric viruses features:

- Diagnostic problems: uncorrelation with faecal bacteria and fagi.
- Norovirus: non culturable > infectivity?
- Low amount in seafood
- Treatment chlorine resistant
- Resistant until 130 days in sea water

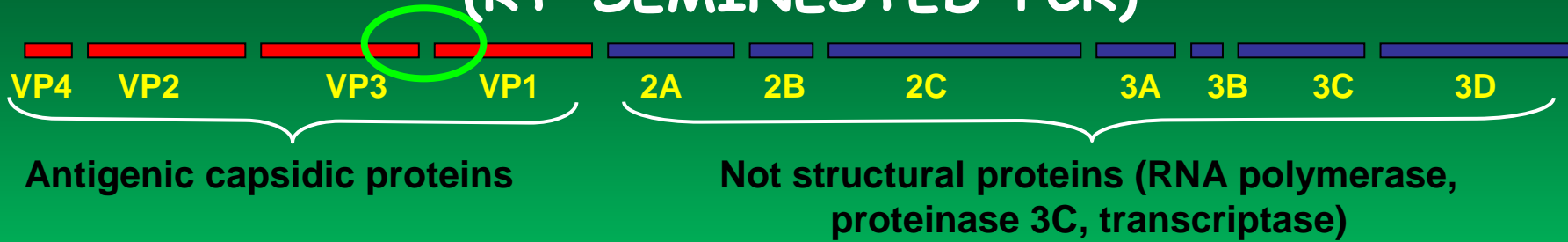
The most common symptoms of **hepatitis A** are fatigue, nausea and/or vomiting, low fever, loss of appetite, dark urine, rash, and jaundice (yellowing of the skin).

Symptoms and signs of hepatitis A can range from none to minimal in the early stages of the illness, to noticeable nausea, abdominal pain, fever, and malaise in the acute phase.

The incubation period is two to six weeks after infection.

Hepatitis A is considered an acute condition.

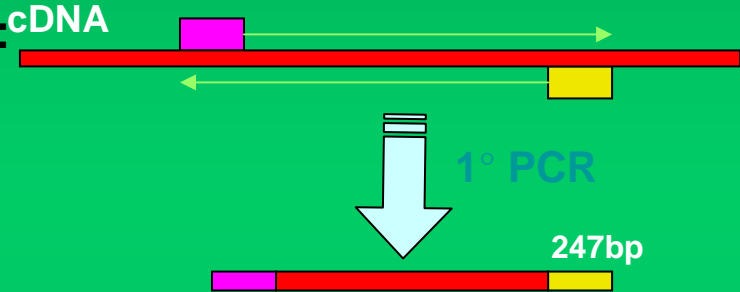
HAV DETECTION (RT-SEMINESTED-PCR)



1^oPCR (*F. Le Guyader et al. 1994*) (247 base pair): cDNA

AV1: 5' GGAAATGTCTCAGGTACTTTCTTTG 3'

AV2: 5' GTTTTGCTCCTCTTTATCATGCTATG 3'



Seminested-PCR (*F. Le Guyader et al. 1994*) (210 bp)

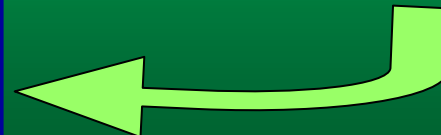
AV2: 5' GTTTTGCTCCTCTTTATCATGCTATG 3'

AV3: 5' TCCTCAATTGTTGTGATAGC 3'



Primers couple AV2/AV3 amplificate a sequence of 210bp, in ORF position translating portion **VP3-VP1**

(position 2167-2377 ca)



The symptoms of **norovirus** illness usually include nausea, vomiting, diarrhea, and some stomach cramping.

Sometimes people additionally have a low-grade fever, chills, headache, muscle aches, and a general sense of tiredness.

The illness often begins suddenly, and the infected person may feel very sick.

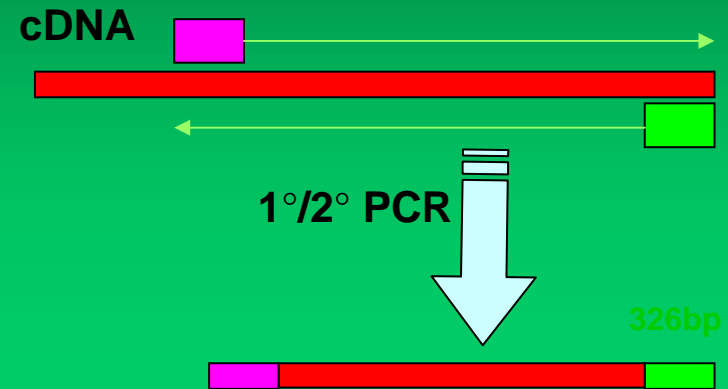
In most people the illness is self-limiting with symptoms lasting for about 1 or 2 days. In general, children experience more vomiting than adults. Most people with norovirus illness have both of these symptoms.

NOROVIRUS DETECTION (RT-BOOSTER PCR)

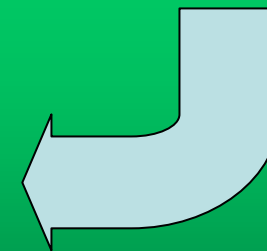
PCR : (J. Vinjè et al. 1997) (326 bp)

JV12Y: 5' ATACCACTATGATGCAGAYTA 3'

JV13I: 5' TCATCATCACCATAGAAIGAG 3'



Primers couple Ni/E3 amplificate a sequence
of 114bp in ORF1 position that translate
for RNA-Polymerase



Real time PCR (HAV and NV)

Primers e probes

NOROVIRUS GI

p1	QNIF4 (FW):	CGC TGG ATG CGN TTC CAT
p2	NV1LCR (REV):	CCT TAG ACG CCA TCA TCA TTT AC
Probe	NVGG1p (PROBE):	TGG ACA GGA GAY CGC RAT CT

NOROVIRUS GII

p1	QNIF2 (FW):	ATG TTC AGR TGG ATG AGR TTC TCW GA
p2	COG2R (REV):	TCG ACG CCA TCT TCA TTC ACA
Probe	QNIFS (PROBE):	AGC ACG TGG GAG GGC GAT CG

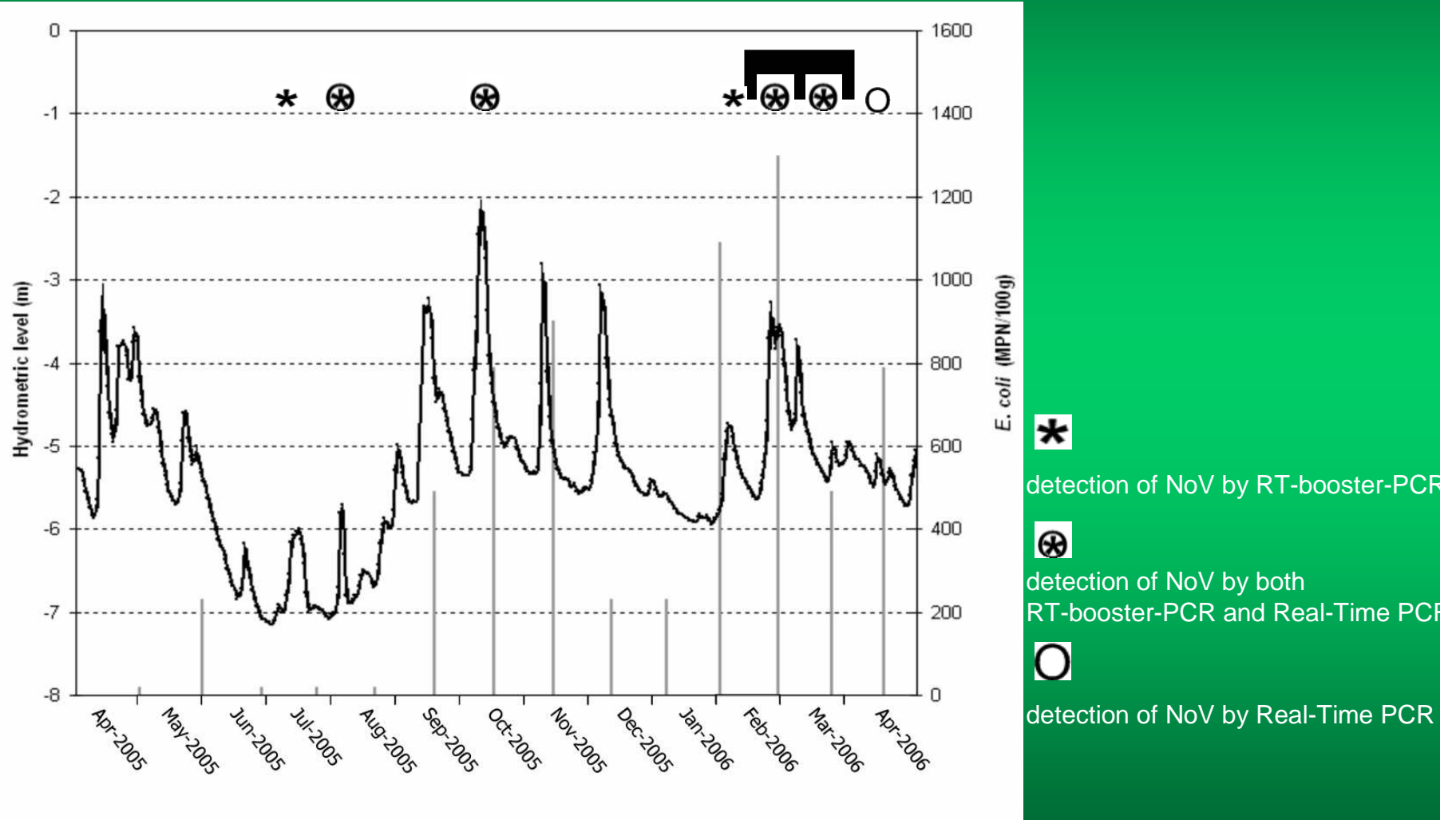
HAV

p1	HAV68 (FW):	TCA CCG CCG TTT GCC TAG
p2	HAV240 (REV):	GGA GAG CCC TGG AAG AAA G
Probe	HAV150(-) (PROBE):	CCT GAA CCT GCA GGA ATT AA

Probe marker: 5' 6-carboxyfluorescein (FAM), 3' MGB (minor groove binder)

Norovirus investigations in lagoon.

Correlation between hydrometric level of the Po river, *E. coli* levels and NoV contamination



Letters in applied microbiology.....Suffredini, Arcangeli, Croci *et al.*...in press

PROTOZOI PATOGENI IDRODIFFUSI NEI PAESI INDUSTRIALIZZATI

Protozoi	Resistenza al Cl ₂	Effetti sulla salute	Diffusione nelle acque	Persistenza in acqua	Epidemie note
<i>Cryptosporidium</i> spp.	Alta	Diarrea acuta e cronica	Comune	Lunga	Sì
<i>Giardia</i> spp.	Alta	Diarrea e malassorbimento	Comune	Moderata	Sì
<i>Toxoplasma gondii</i>	?	Linfoadenopatia, febbre, infezioni congenite	?	?	Sì
<i>Microsporidia</i>	?	Diarrea e perdita di peso	?	?	Sì
<i>Cyclospora cayetanensis</i>	?	Diarrea persistente	?	?	Sì

(Carraro *et al.*, 2004)

DIRECT IMMUNOFLUORESCENCE:

MeriFluor® :

- Identification reagent: monoclonal antibodies anti-oocystis of *Cryptosporidium* and anti-cistis of *Giardia* linked with FITC (Fluoresceine isotiocyanate)



Possibility to adopt alternative microbiological methods (validated according ISO EN 16140)

➤ Organising laboratories

- ✓ CRL
- ✓ French NRL

➤ Participating laboratories

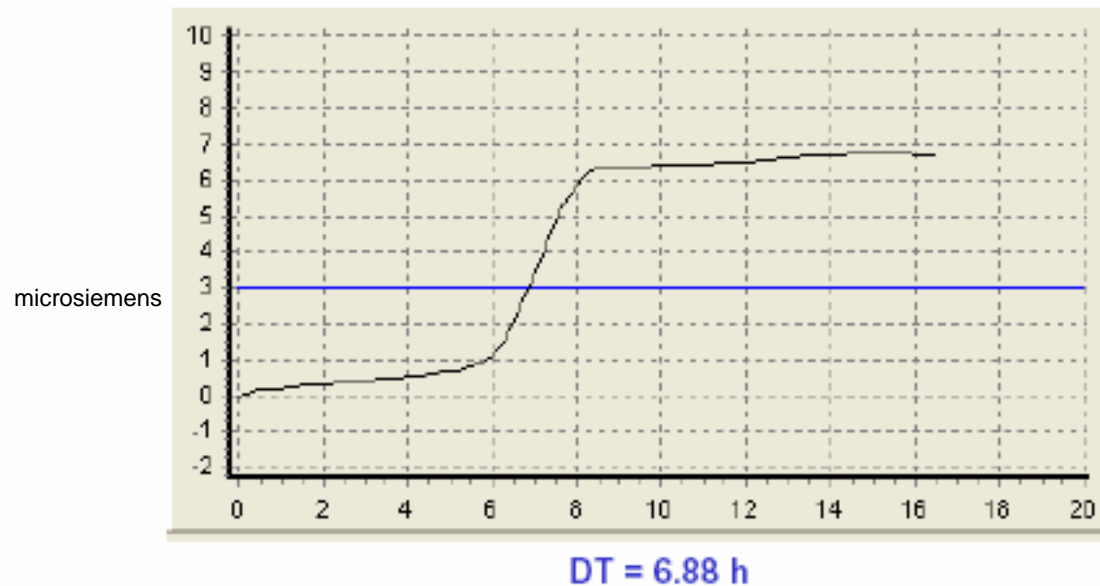
- ✓ 8 French laboratories : 6 Ifremer (NRL, Normandie, Morbihan Pays de la Loire, Pertuis Charentais, Languedoc Roussillon), Departmental Veterinary Laboratory Saint Lô (Normandie), DVL La Rochelle (Poitou Charentes), DVL Montpellier (Languedoc Roussillon)
- ✓ 4 European laboratories : NRL U.K. (CEFAS, Weymouth), NRL Austria (AGES, Vienna), Italy (Istituto zooprofilattico delle Venezie) and Spain (INTECMAR, Galicia)

Impedometric system



Impedance measurement: through the detection of electric conductivity changing in colture medium

1 *Escherichia coli* typical impedance curves

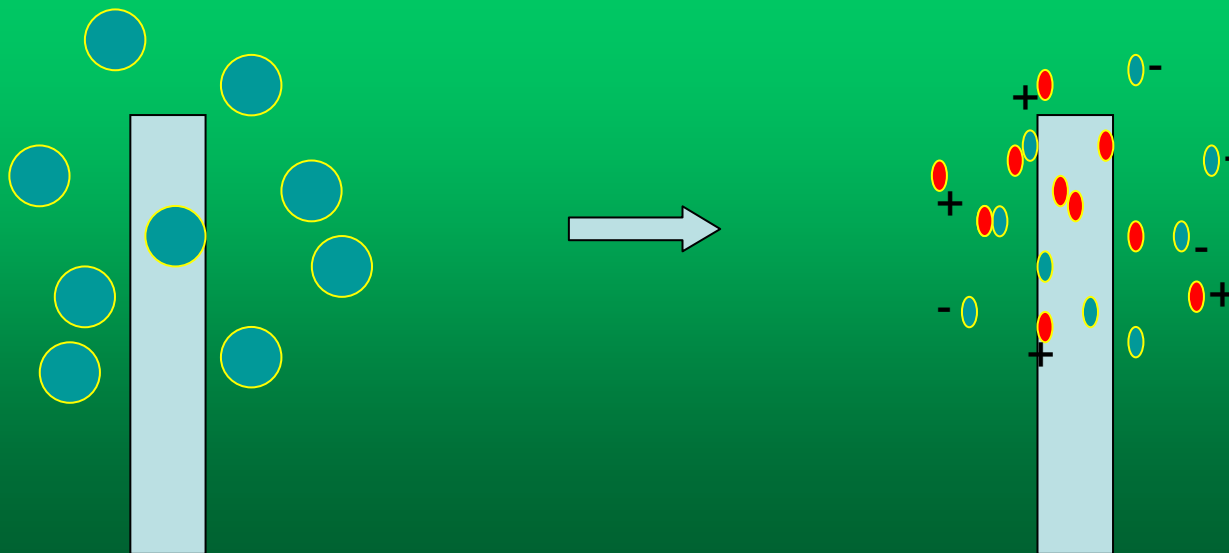


Detection time (DT): needed time to assess a significant conductivity variation
Depends from the examined strain, cellular and colture medium composition

Impedance measurements

Conductance: assess the increase of conductivity in the medium
(opposite of resistance)

Capacitance : record the accumulation of electric charges followed
by the increasing of ionic concentration in proximity of the electrodes



Int. J. Food Microbiol. 105 (2005), 139-144

***Resistance of hepatitis A virus in mussels
subjected to different domestic cookings***

Luciana Croci, Dario De Medici, Simona Di Pasquale and Laura Toti

**Istituto Superiore di Sanità,
Centro Nazionale per la Qualità degli Alimenti ed i Rischi Alimentari,
Viale Regina Elena, 299 00161, Rome, Italy**

VONGOLE VERACI (*Tapes philippinarum*)

Paese di spedizione Italia - Prodotto allevato Italia.
Peso da riscontrare alla vendita (tara 10 g.)
purchè conservato alla temperatura di 0+6°C.
Questi animali devono essere vivi al momento
dell'acquisto. **Da consumarsi previa cottura.**
Data di confezionamento impressa sul retro



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