ENTERIC VIRUSES AS EMERGING RISKS IN FOOD SAFETY

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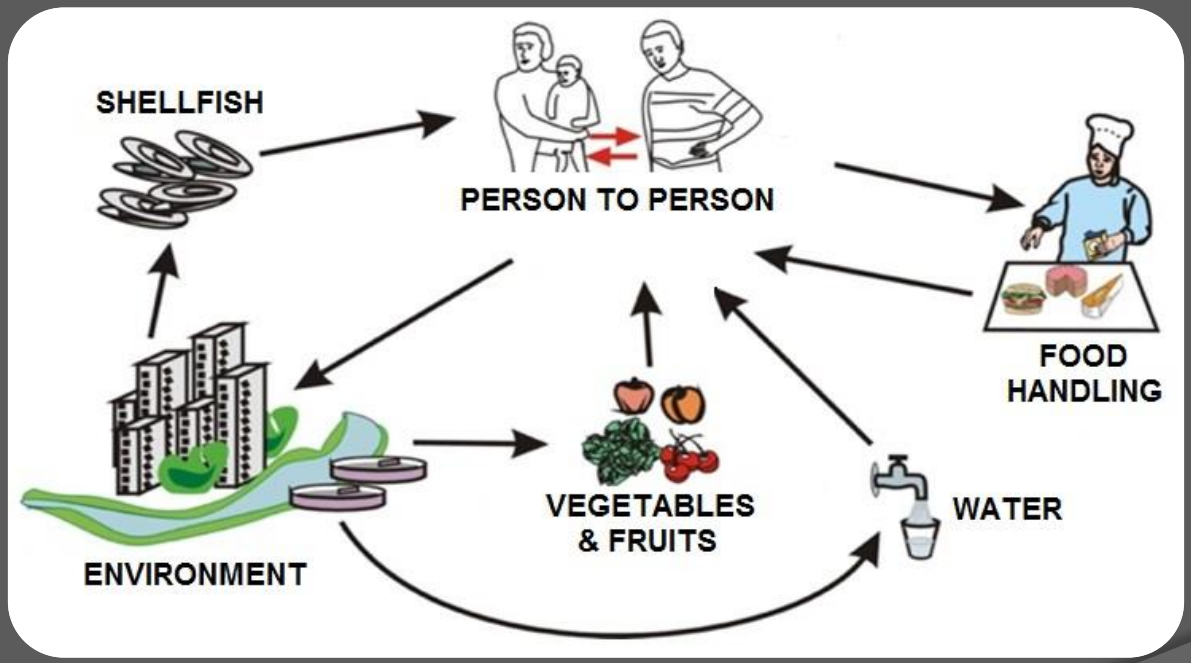
1. INTRODUCTION

Transmission → *FECAL-ORAL ROUTE*



Molluscs act as vectors of viral gastroenteritis and other diseases as they concentrate and retain pathogens, some of them being endemic in developing countries.

1. INTRODUCTION

Evaluation of emerging risks (climate change, aging, etc.), and development of preventive measures

Totally new risks

New risks by the kind of product

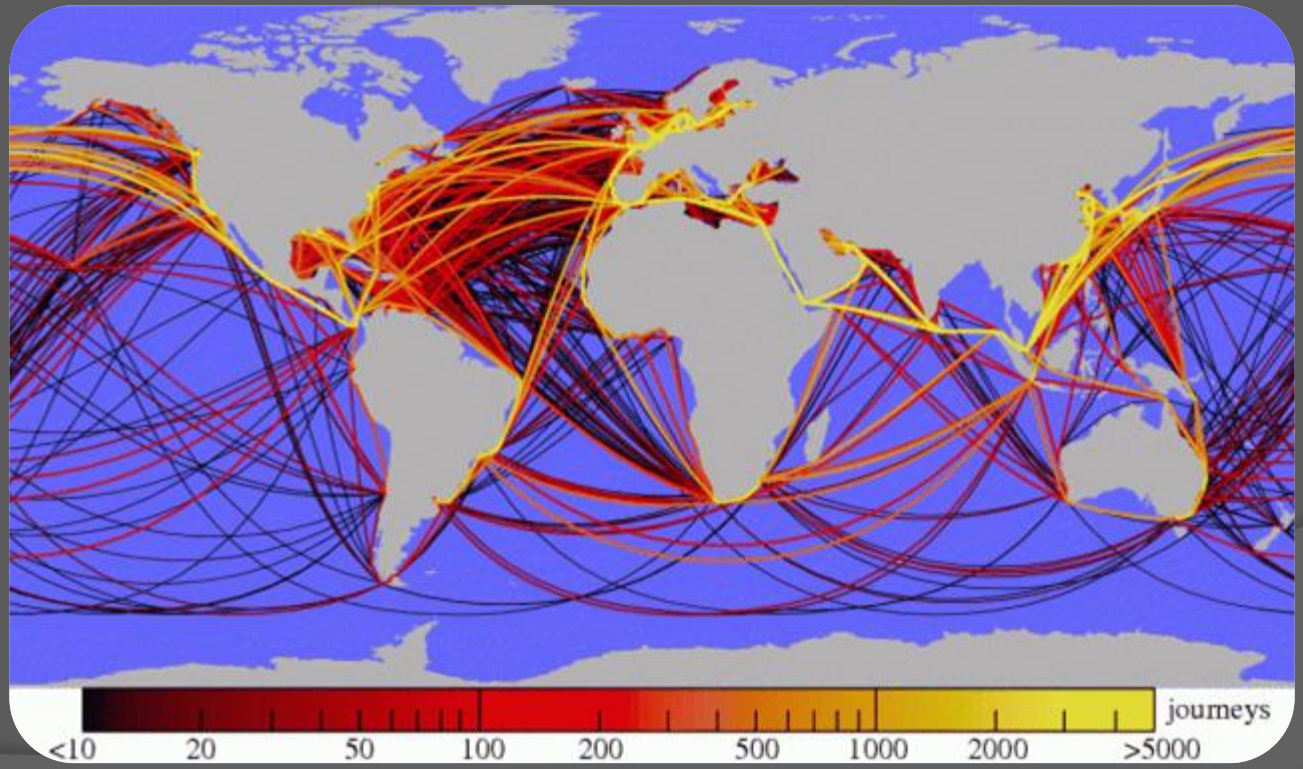
New risks for specific geographic areas

Risks diminished for areas geographic specific

Risks increased for areas geographic specific

1. INTRODUCTION

Globalization has increased the risk for infectious foodborne diseases due to the international trade of food products, including bivalve molluscs.



1. INTRODUCTION

Int Microbiol (2001) 4: 223–226
DOI 10.1007/s10123-001-0041-0

RESEARCH ARTICLE

J.L. Romalde · I. Torrado · C. Ribao · J.L. Barja

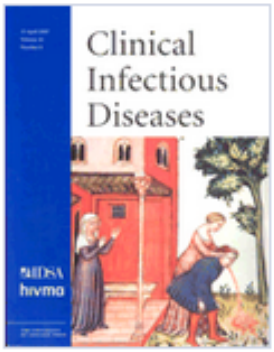
Global market: shellfish imports as a source of reemerging food-borne hepatitis A virus infections in Spain

Water Sci Technol. 2001;43(12):61-5.

Human enteric viruses in Coquina clams associated with a large hepatitis A outbreak.

Bosch A[1], Sánchez G, Le Guyader F, Vanaclocha H, Haugarreau L, Pintó RM.

1. INTRODUCTION



Volume 44, Issue 8
15 April 2007

Internationally Distributed Frozen Oyster Meat Causing Multiple Outbreaks of Norovirus Infection in Australia FREE

R. J. Webby ✉, K. S. Carville, M. D. Kirk, G. Greening, R. M. Ratcliff, S. K. Crerar, K. Dempsey, M. Sarna, R. Stafford, M. Patel, ... Show more

Clin Infect Dis (2007) 44 (8): 1026-1031.
DOI: https://doi.org/10.1086/512807

N Z Med J. 2007 Oct 26;120(1264):U2773.

A New Zealand outbreak of norovirus gastroenteritis linked to the consumption of imported raw Korean oysters.

Simmons G[1], Garbutt C, Hewitt J, Greening G.

1. INTRODUCTION

Novel enteric viruses have emerged as responsible for food-borne outbreaks associated with this type of food, or have been detected in meat, shellfish, fruits, etc.

Aichi virus (AiV)	Sapovirus (SaV)
Family *Picornaviridae*	Family *Caliciviridae*
Genus *Kobuvirus*	Genus *Sapovirus*
Icosahedral morphology	Icosahedral morphology
Non-enveloped	Non-enveloped
Genome 8.4 Kb (ssRNA)	Genome 7.5 Kb (ssRNA)

Hepatitis E virus (HEV)
Family *Hepeviridae*
Genus *Orthohepevirus*
Icosahedral morphology
Non-enveloped
Genome 7.2 Kb (ssRNA)

2. OBJECTIVES

The detection and quantification of emerging enteric viruses (AiV, SaV and HEV) in shellfish samples from harvesting areas in Galicia (NW Spain).

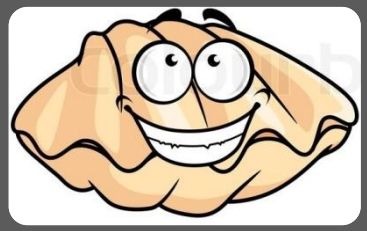
Detection of enteric viruses in clinical samples. Epidemiology.

The detection and quantification of Aichi virus (AiV) and Sapovirus (SaV) from shellfish samples imported to Spain.

The efficiency of depuration in the elimination of enteric viruses from shellfish.

3. MATERIAL & METHODS

PROCESING & EXTRACTION

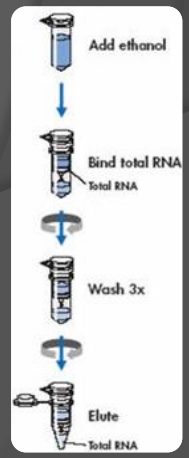


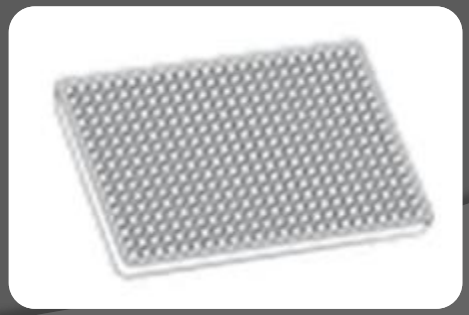
- Homogenization from digestive tissue
- RNA extraction: NucleoSpin RNA Virus Kit

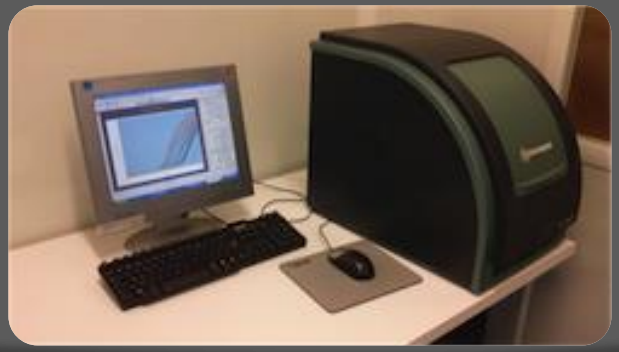
ISO/TS/15216:2013 / 2017

DETECTION & QUANTIFICATION

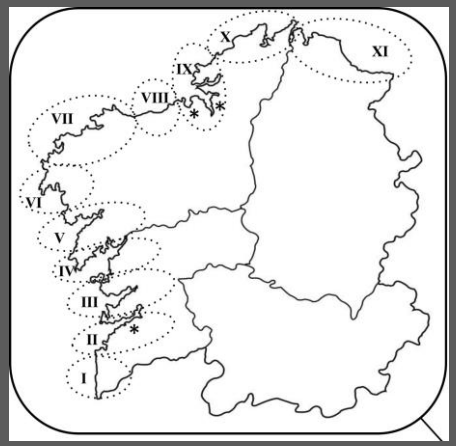
- RT-qPCR with specific primers and TaqMan probes,
- Standard curve: serial dilutions of cloned fragment in plasmid

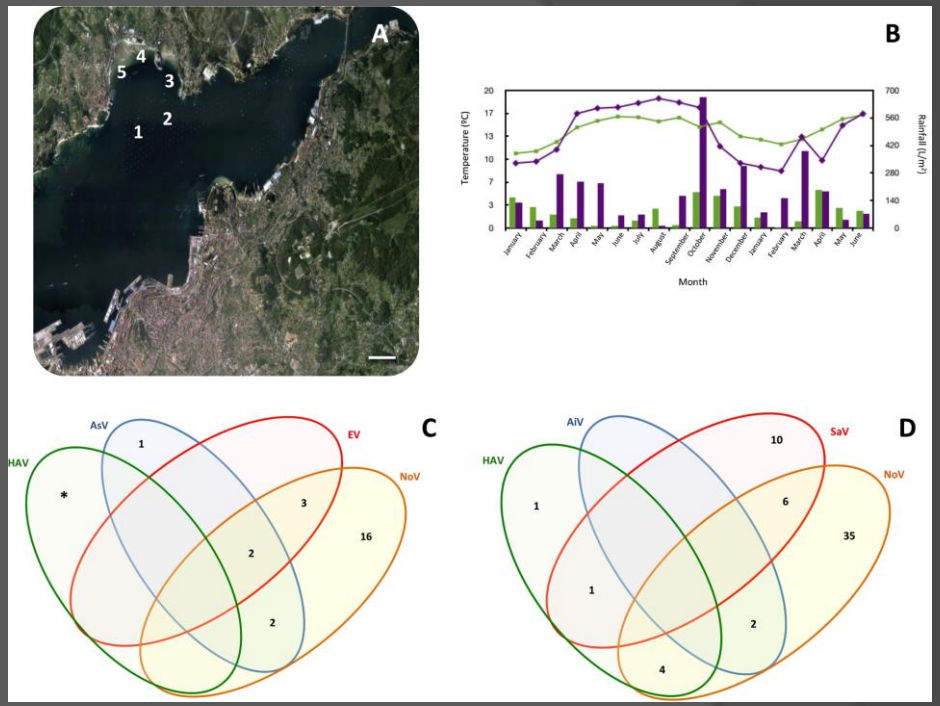


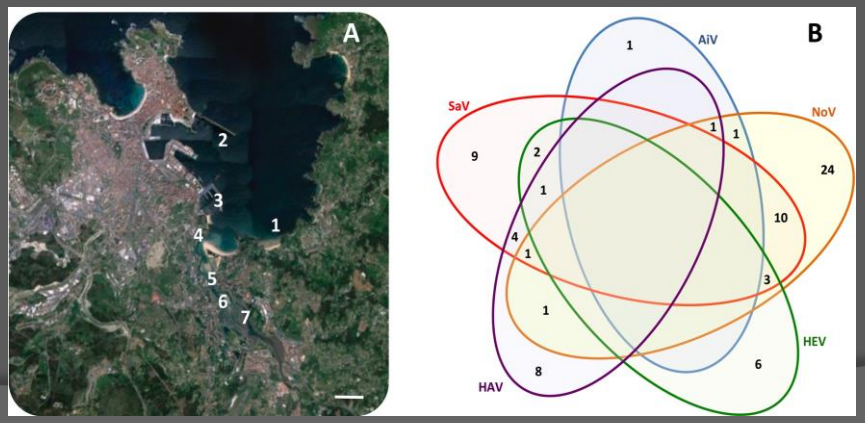


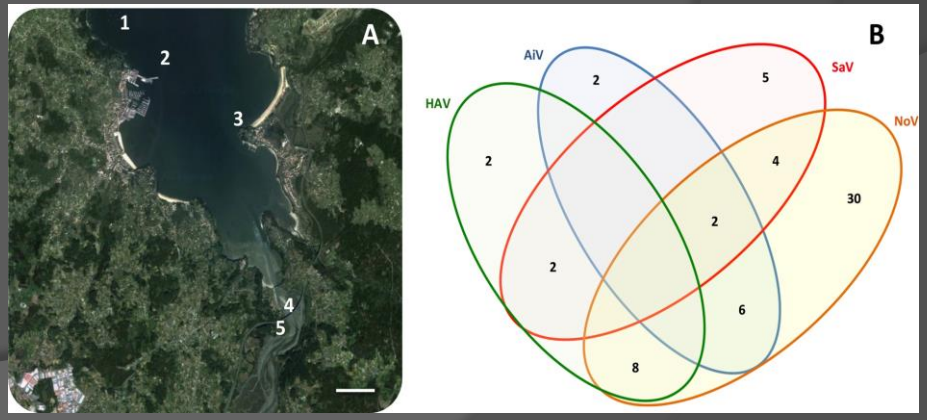


4. RESULTS

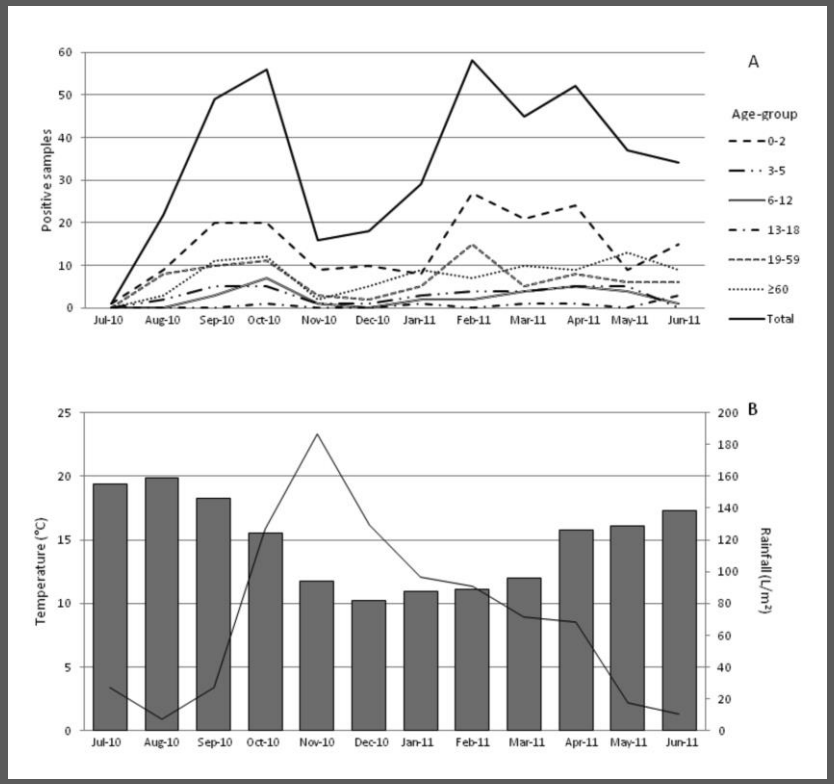

I
II
III
IV
V
VI
VII
VIII
IX
X
XI

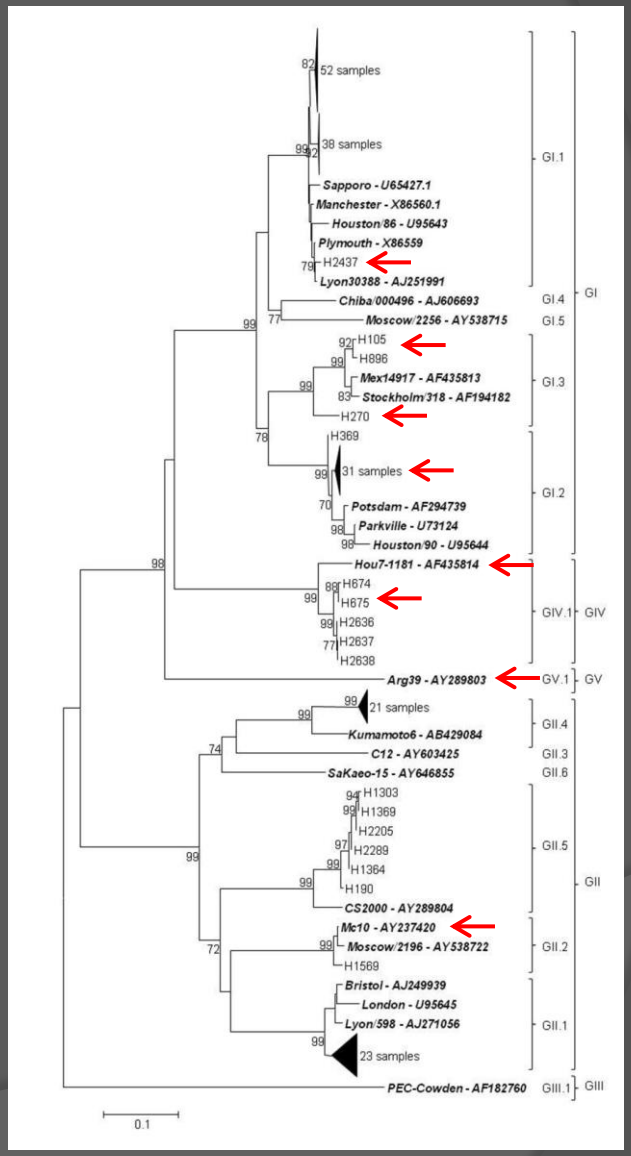

A
B
Temperature (ºC)
Rainfall (L/m²)
Month
C
AsV
EV
HAV
NoV
D
AiV
SaV
HAV
NoV


A
B
AiV
SaV
NoV
HEV
HAV


A
B
AiV
SaV
HAV
NoV

4. RESULTS


A
Positive samples
Age-group
0-2
3-5
6-12
13-18
19-59
≥60
Total
Jul-10
Aug-10
Sep-10
Oct-10
Nov-10
Dec-10
Jan-11
Feb-11
Mar-11
Apr-11
May-11
Jun-11
B
Temperature (°C)
Rainfall (L/m²)


52 samples
38 samples
Sapporo - U65427.1
Manchester - X86560.1
Houston/86 - U95643
Plymouth - X86559
H2437
Lyon30388 - AJ251991
Chiba/000496 - AJ606693
Moscow/2256 - AY538715
H105
H896
Mex14917 - AF435813
Stockholm/318 - AF194182
H270
H369
31 samples
Potsdam - AF294739
Parkville - U73124
Houston/90 - U95644
Hou7-1181 - AF435814
H674
H675
H2636
H2637
H2638
Arg39 - AY289803
21 samples
Kumamoto6 - AB429084
C12 - AY603425
SaKaeo-15 - AY646855
H1303
H1369
H2205
H2289
H1364
H190
CS2000 - AY289804
Mc10 - AY237420
Moscow/2196 - AY538722
H1569
Bristol - AJ249939
London - U95645
Lyon/598 - AJ271056
23 samples
PEC-Cowden - AF182760
GI.1
GI.4
GI.5
GI.3
GI.2
GIV.1
GV.1
GII.4
GII.3
GII.6
GII.5
GII.2
GII.1
GIII.1
GI
GIV
GV
GII
GIII
0.1

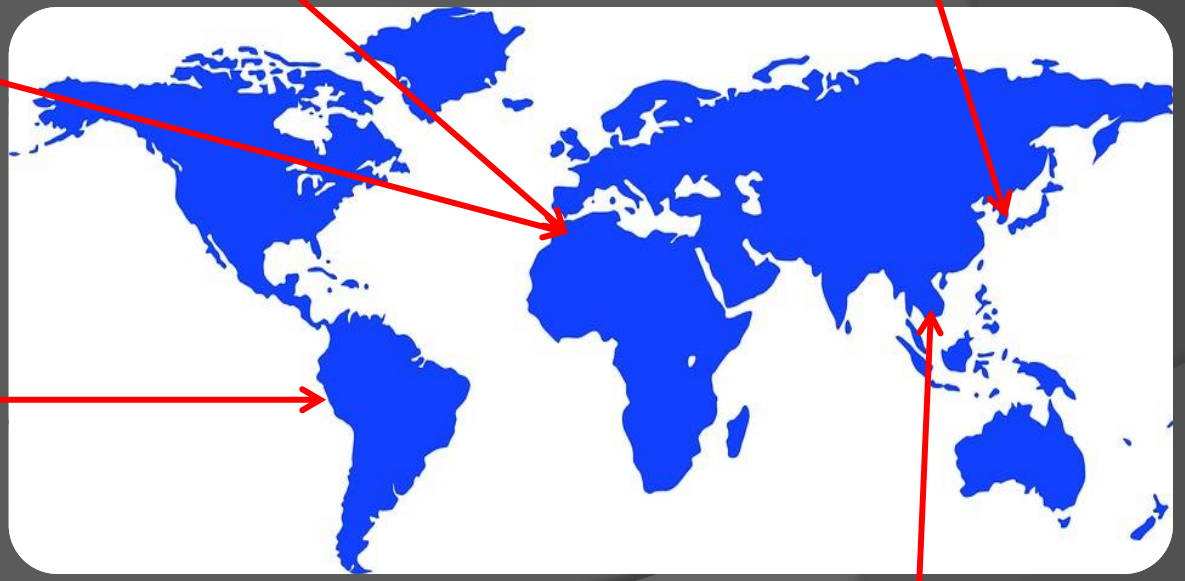
4. RESULTS

Total of 54 shellfish samples imported between Sep-2006 and Jan-2011

City of Melilla (Spain)
Clams (n=2) and mussels (n=1)

South Korea
Meretrix lyrata n=1

Morocco
Callista chione n=21
Ensis sp. n=6
Donax sp n=2
Crassostrea angulata n=1
Cerastoderma edule n=1
Solen marginatus n=1



Peru
Transanella pannosa n=6
Argopecten purpuratus n=5
Donax sp n=3
Ensis sp. n=2

Vietnam
Meretrix lyrata n=2

4. RESULTS

SaV was detected in 29 samples

SaV	DETECTION							QUANTIFICATION cRNA/g DT	
	TOTAL	Clams	Oysters	Mussels	Scallops	Cockles	Razor clams	Average	Range
TOTAL	***53.7 %***	***43.2 %***	***100 %***	***100 %***	***100 %***	***100 %***	***55.6 %***	**1.2×10^5**	**$1.4 \times 10^3 – 9.9 \times 10^6$**
Morocco	***37.5 %***	21.7 %	100 %			100 %	71.4 %	6.0×10^4	$1.4 \times 10^3 - 9.9 \times 10^6$
Peru	***68.8 %***	66.7 %			100 %		0 %	2.3×10^5	$1.3 \times 10^4 – 3.2 \times 10^6$
Vietnam	***100 %***	100 %						4.8×10^4	$1.8 \times 10^4 – 1.3 \times 10^5$
S. Korea	***100 %***	100 %						-	-
Melilla	***100 %***	100 %		100 %				2.5×10^5	$2.5 \times 10^4 – 1.7 \times 10^6$

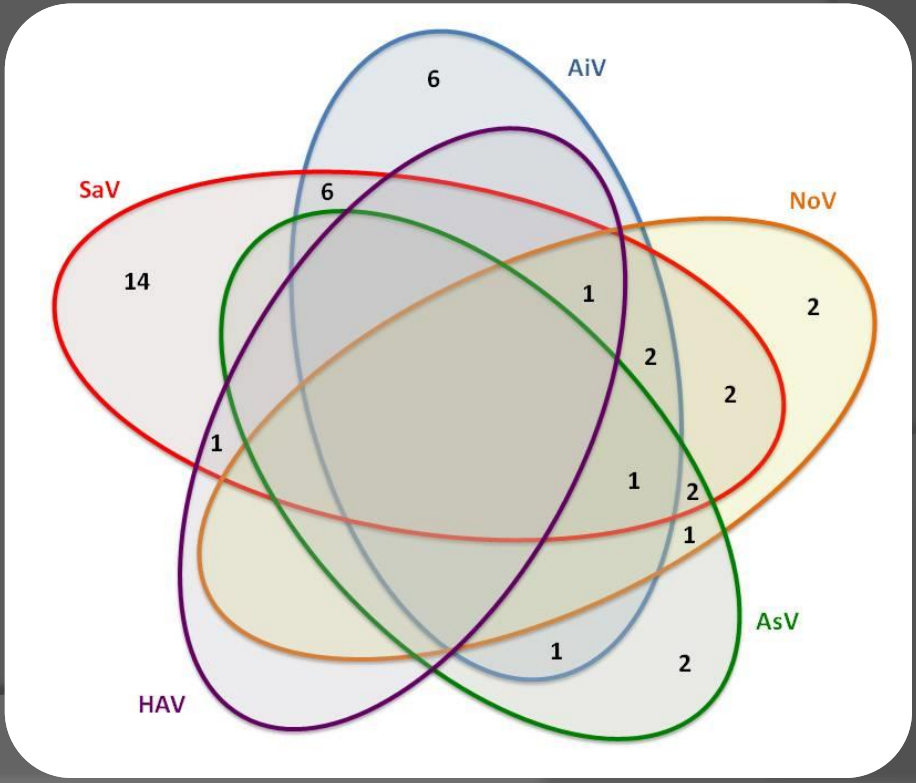
AiV was detected in 18 samples

AiV	DETECTION							QUANTIFICATION cRNA/g DT	
	TOTAL	Clams	Oysters	Mussels	Scallops	Cockles	Razor clams	Average	Range
TOTAL	***33.3 %***	***35.1 %***	***0 %***	***0 %***	***0 %***	***100 %***	***44.4 %***	**2.4×10^4**	**$4.9 \times 10^2 – 3.6 \times 10^6$**
Morocco	***37.5 %***	34.8 %	0 %			100 %	42.9 %	3.9×10^4	$1.6 \times 10^3 - 3.6 \times 10^6$
Peru	***18.8 %***	22.2 %			0 %		50.0 %	2.8×10^4	$1.5 \times 10^4 - 5.9 \times 10^4$
Vietnam	***50.0 %***	50.0 %						-	-
S. Korea	***100 %***	100 %						-	-
Melilla	***33.3 %***	50.0 %		0 %				-	-

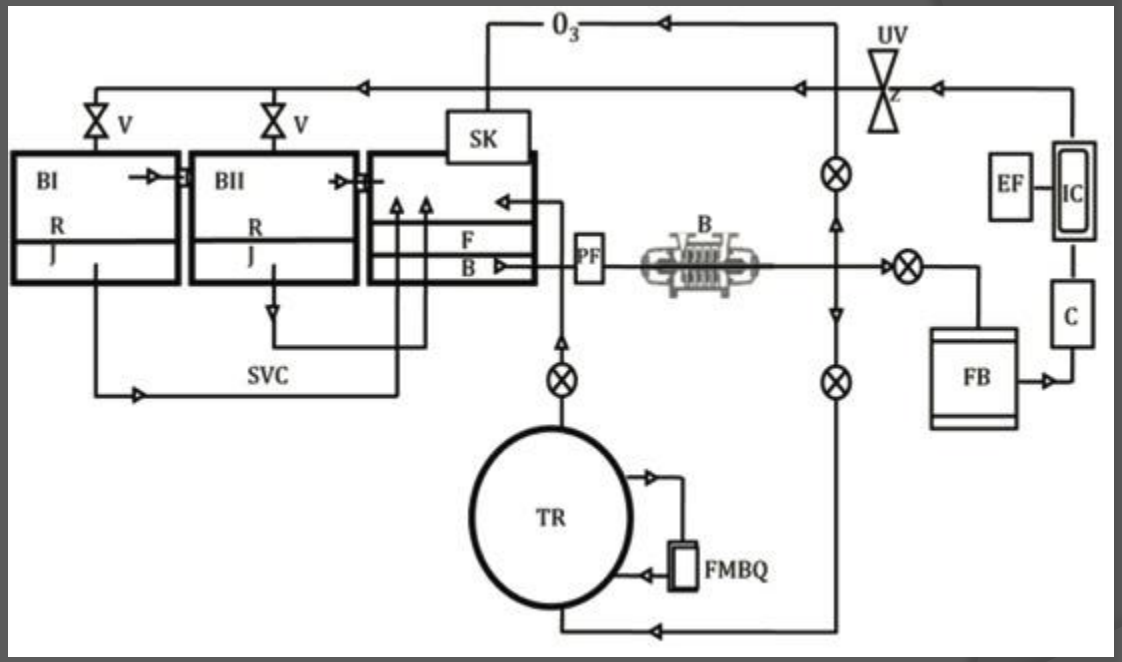
4. RESULTS

Mixed contamination with AiV and SaV was detected in 10 samples. It occurred in all countries but especially in Morocco (5).

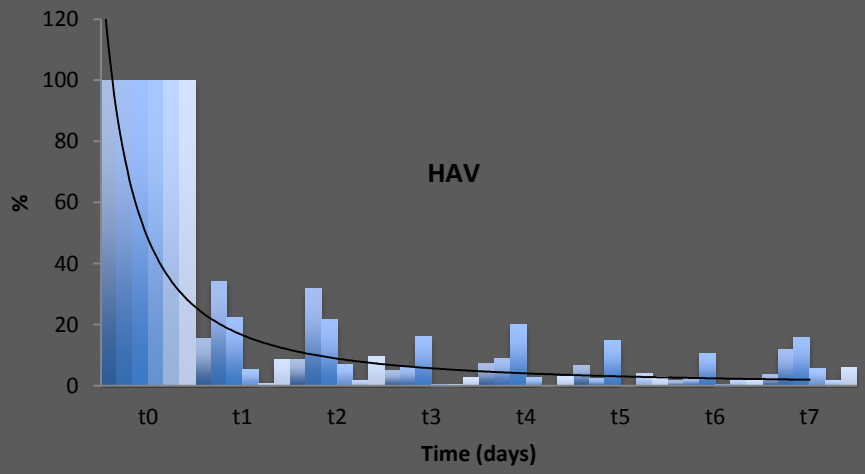
Results compared with previous studies of Norovirus (NoV), hepatitis A virus (HAV) and Astrovirus (AsV).

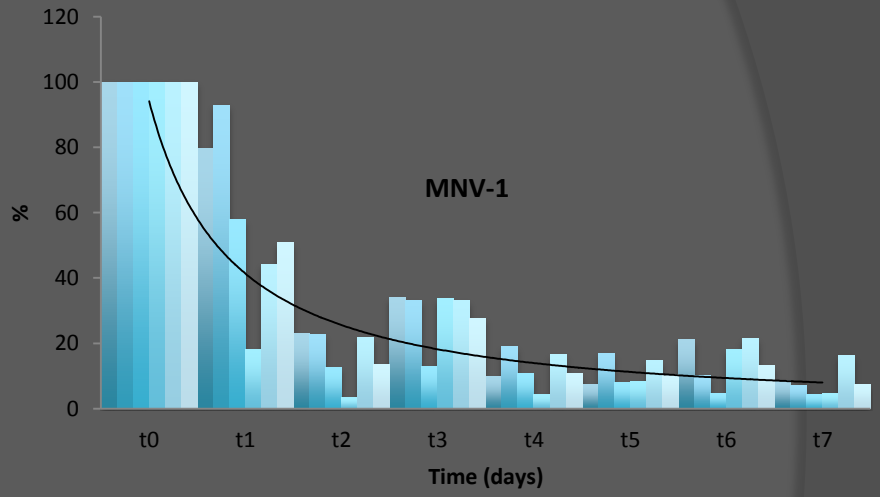


Experimental depuration system



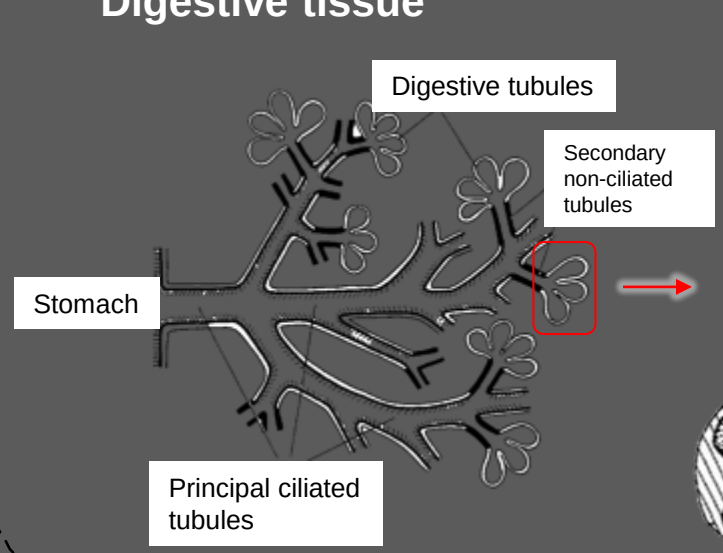
- Close circuit (ASE M BINS)
- 500 kg of capacity
- Water volume 1,750 L
- Water disinfection by O_3 y UV
- Mechanic, chemical and biological filter

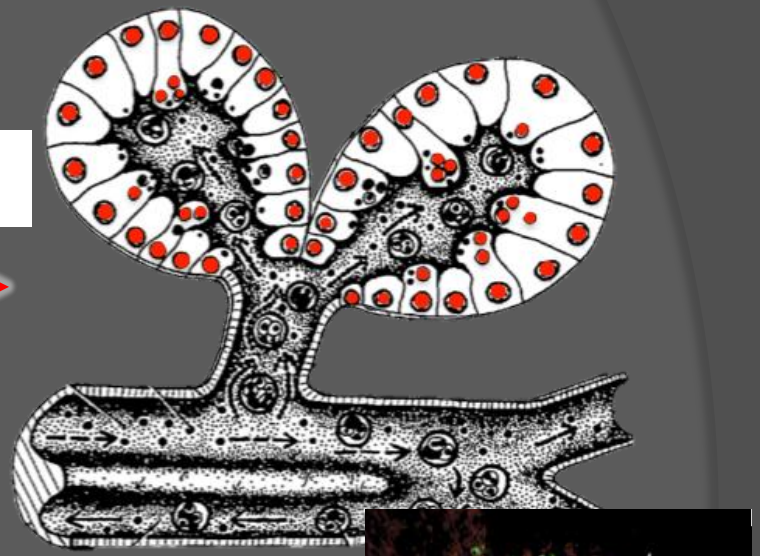


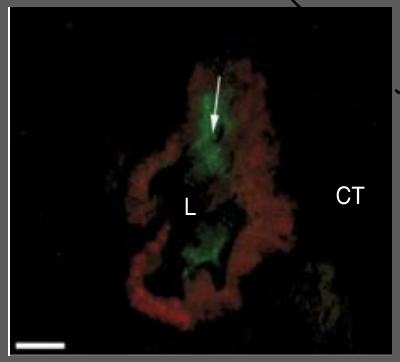


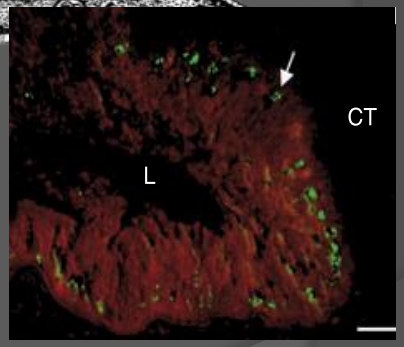
- **Different mollusks showed different depuration rates**
- **Always a two-phases kinetic**

Digestive tissue





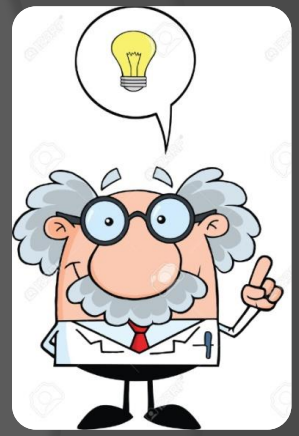




$$c(t) = c_r + (1 - c_r)\, e^{-\lambda t}$$

5. CONCLUSIONS

• It is important to continue the studies with shellfish and clinical samples in order to detect shifts in the major viral groups/genotypes, and to try to determine the vehicles of transmission.



• A great variety of mixed contaminations are observed, both in Galician harvesting areas and in imports, especially when comparing these results with previous studies for Norovirus and hepatitis A virus.

•Further depuration studies are needed with the known and emerging viruses.

• Further research is needed in order to determine the importance of emerging enteric viruses, as SaV, HEV and AiV, as cause of possible foodborne outbreaks associated to shellfish.

Moitas grazas pola vosa atención !!