Developing a strategy to limit shellfish viral contamination

Le Saux J.C.[1*], Dérolez V[1], Brest G[2]., Le Guyader F[1] and M Pommepuy[1]
[1] Ifremer, centre de Brest, Plouzané, France; [2] CNC, Paris, France.
*jean.claude.le.saux@ifemer.fr

Introduction

For shellfish, the viral contamination clearly occurs in the first step of the process *i.e.* in growing and harvesting areas. As opposed to other foods, there is no proof that other routes (foodhandlers, aerosol), could be at the origin of the seafood contamination (Koopmans & Duizer, 2004). Most of the time, untreated sewage are at the origin of the presence of viruses in shellfish. However, direct contamination by ill people working in the growing area, has also been reported (Berg *et al.*, 2000; Butt *et al*, 2004).

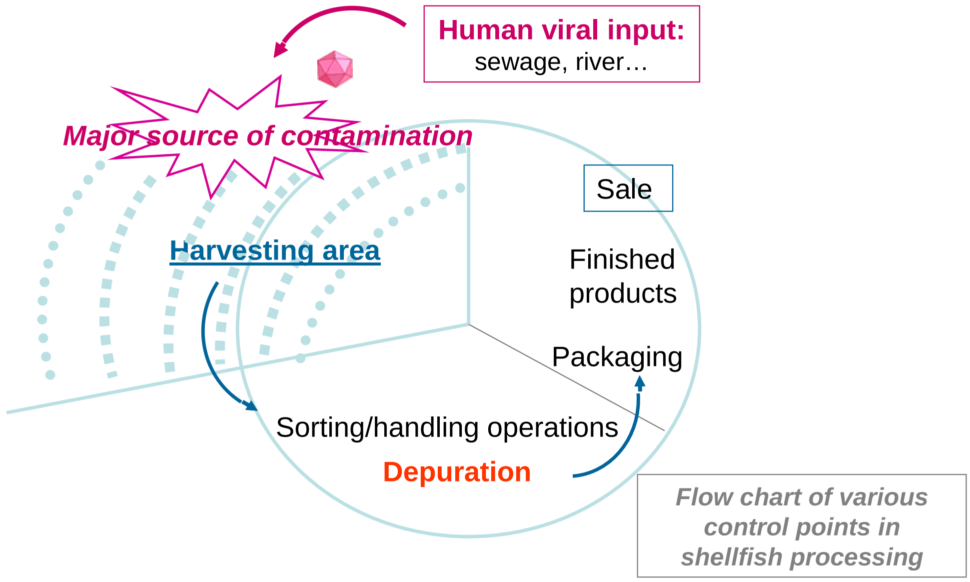

Figure 1: Proven route of shellfish contamination during the process and HACCP in shellfish processing.

The presence of pathogens in the environment mainly depends on the density of the coastal urban and animal populations. Seasonal outbreaks occurrence or rainfall input, also contributes in modifying the load of discharged pathogens. During the epidemic period, a large viral input can be spread in the environment from sewage networks or inefficient sewage treatment plants (Miossec *et al*, 1998). Some years are favourable to winter epidemics in the population. For example, during the last main outbreak in France, 6 million people became ill over a 6-weeks period (January 2000 - February 2001). If we consider an attack rate equivalent to 3% of the population, and a viral concentration in faeces of about 30 million particles/ patient, the viral flux from 15 000 inhabitants could be estimated to 600 000 viruses/per minute (Pommepuy *et al*, 2004). Associated with forecast events (rainfall) or any failure in the sewage network, this could be at the origin of a viral shellfish contamination.

Epidemics are recorded in the human population by European Networks (Réseau Sentinelle – France, RIVM - The Netherlands, PHLS - UK…etc). Currently web sites inform the presence of epidemic in the population (ex: Sentiweb, www.b3jussieu.fr. These sources of information are interesting for shellfish quality surveillance and could participate to define "week or month risk" in a risk assessment procedure.

Viruses persist in seawater and shellfish for a long time (weeks or months) (Wait & Sobsey, 2001; Bosch, 1995). Depuration, which is a procedure included in HACCP processing cannot solve the problem with the present depuration systems using seawater at local water temperature (< 12°C during epidemic months) (Lees, 2000; Doré & Lees, 1995, Sobsey & Jackus, 1991). However, depuration based on shellfish staying - for a long period in tanks equipped with increase seawater temperature (20-25°C) - could be able few cases and under specific conditions (low contamination, recent contamination), to partially solve the problem (Pommepuy *et al*, 2004; Doré *et al*, 2004). But in any case, this can be a real warranty to have safe products. This is due to the persistence of some species of viruses, the lack of information concerning the undesirable indirect effects at this temperature (Vibrio growth). Moreover, this process is not adapted for all species of shellfish (clams, mussels, cockles…) and important mortalities are observed for shellfish submitted to these temperatures. This process has to be used in exceptional circumstances only when there is a known enteric virus risk. Because of this, we suggest a further investigation in harvesting area to preserve water quality from microbial contamination, rather than to only focus on shellfish depuration.

How to limit the risk?

1. Tracking the sources

Rivers are the major routes, from the land to the sea, for the natural products of weathering and many man-hand materials and they play a general role of pathways of materials from the land to the ocean (Crowther *et al*, 2001, 2002) .

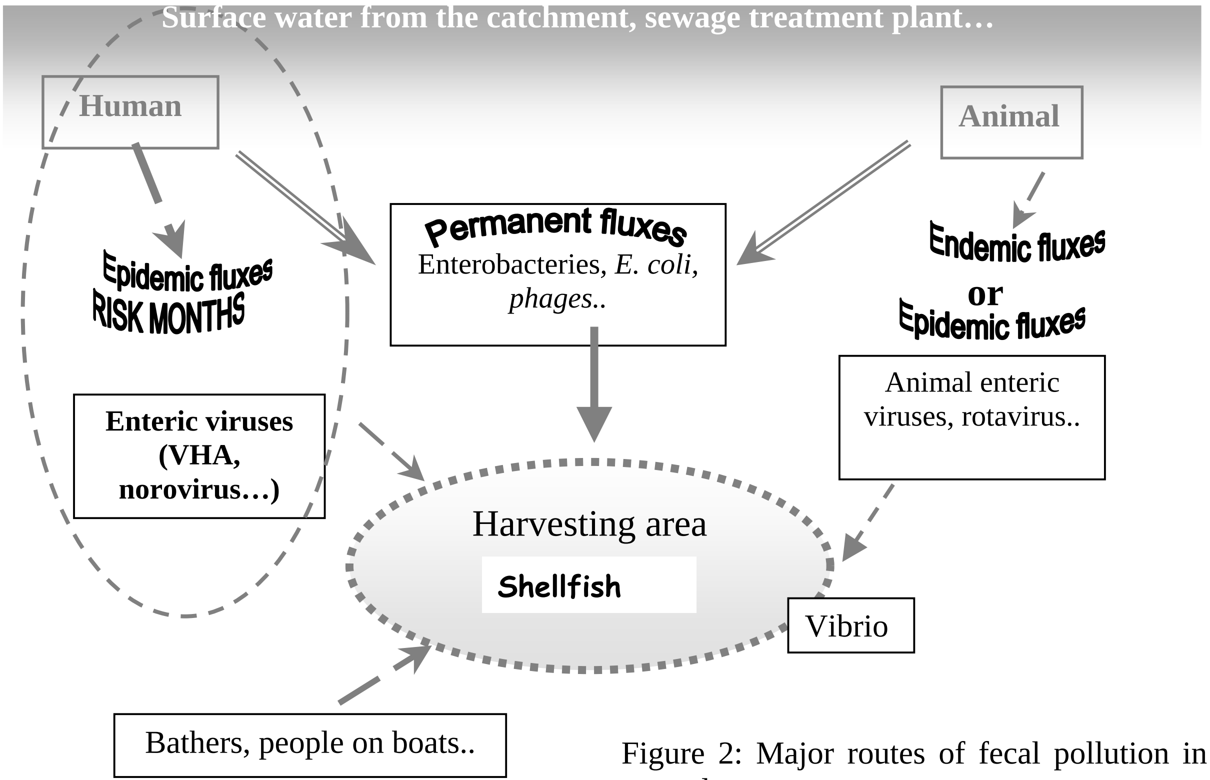

Figure 2: Major routes of fecal pollution in coastal areas.

Three different types of faecal fluxes exist and are presented on figure 2. Permanent faecal fluxes from intestinal flora (estimated with *E. coli* and *Enterococcus)*, epidemic pathogenic fluxes resulting from human or animal outbreaks (ex: viruses) and endemic fluxes (ex: bacterial animal carrying) are susceptible to reach estuaries and coastal waters. Among others sources of contamination, bathers, boats or vessels could also have a significant impact on water and sediment contamination (Gerba, 2000; Sobsey *et al*, 2003). Dowel e*t al*, (1995) also demonstrated that overboard disposal of sewage, currently practiced on harvesting areas could be at the origin of fecal oyster contamination.

Studies have to be undertaken to document all the critical points (*i.e*. the sources of contamination: urban, industrial or agricultural), which can affect the shellfish beds in an harvesting area. Table 1 reports the main factors influencing the microbial quality of water in coastal areas and some examples of possible preventive actions, control or monitoring (Dérolez V, 2003).

	Hazard Analysis	**Parameters to gather**	**Preventive, control or monitoring procedure**
Sewage input (Document the sources: status quo)	Human population	- Density - Age of urban network and STP - Evolution of population - Harbour proximity	Establish the list of main outfall/sources, control network and survey
	Animal population	- Density - Density in the 500m area from shoreline - Evolution of animal pop. - Sewage farm	Control agriculture regulation application in the area and the conformity with law (specially for spreading of manure)
	Network or existing survey	- Open data bases - Real-time data	Assess the evolution of parameters for the last period (ten years)
Factors influencing the microbial quality of the area	River fluxes	- Catchment area (urban or agriculture activities)	Control the local urban scheme and agricultural program.
	STP (capacity, type, age)	- Conformity - Performance	Control standard and application of the regulation
	Network (age, unitary or separatif)	- By-pass - Sewage-raise station - Sewage discharge - Parasite water	application of the regulation Control the discharge
	Marshy shore	- Drain	
	Tide	- Amplitude	Assess the critical periods
Aggrieving factors	Outbreak in population	- Human gastro-enteritis	Survey
	Forecast	- Amplitude - Period, length	Survey

Table 1: Determination of the potential sources which can affect water contamination.

Gathering the data will lead to identify the main viral sources and then, to determine corrective actions to be taken: repairing sewage network, building a new STP with high capacity, biological treatment, deleting illegal sewage outfall in coastal areas...

Risk assessment of harvesting areas to pollution is also links to the shellfish type and the influencing/aggreaving factors of the water quality. Table 2 reports the main potential critical points and the monitoring procedures to be carried out to prevent the quality of this activity.

Parameters	Hazard Analysis	Critical points	Preventive, control or monitoring procedure
Status quo	Activity	- Shellfish species - Table, soil, line	Illness surveillance Silt removal
	Harvesting type	- Growing - Stocking - Laying - Refining	Traceability
	Site description	- Estuary, bay. - Water renewal - Tide amplitude - Shore amplitude - Soil: silt, sand..	Description, Observation
Influencing factors	STP and river input	- Salinity variation, SM - Water stratification - Contaminants - Microbiological quality	Network
	Tide	- Amplitude - Percentage emersion	*In situ* recording
	Rainfall	- Salinity decrease	*In situ* recording
Aggrieving factors	Input	- Salinity decrease	*In situ* recording
	Season	- Low temperature	*In situ* recording
	Urban input	- Viral input	Population survey

Table 2: Critical points and shellfish quality in harvesting areas.

To manage the risk, preventive and corrective actions have to be taken in the event of a virus alert. Depending on the intended destination of batches potentially contaminated different actions have to be recommended (table 3).

Type of stock	Batches intended for	Preventive action	Corrective action
Spat	Transfer	/	/
18 months	Transfer	Traceability	More rigorous traceability
2 years	1. Sale to the public	Virological testing	More rigorous depuration
	2. Transfer	Traceability and Viral testing	Depuration prior to sale
More than 2 years	1. Sale to the public	Viral testing	More rigorous depuration
	2. Transfer	Traceability and Viral testing	More rigorous depuration prior to sale

Table 3: Preventive and corrective actions in the event of a virus alert.

2. Implement an early warning system for shellfish production sites

Contamination could occur even when corrective actions reduced the risk: forecast event, for example could occur and be at the origin of sewage run-off, STP could also have failures... etc. To avoid shellfish to be contaminated, necessary control points have to be surveyed and an early warning system could be implemented in coastal areas (Figure 2). Different parameters could be recorded (rainfall, salinity, sewage network key-points, epidemic…) and gathered in a data base. Particular event able to decrease water quality should thus be detected and shellfish producers immediately informed (ex: figure 2, alarm from Point 2.bis).

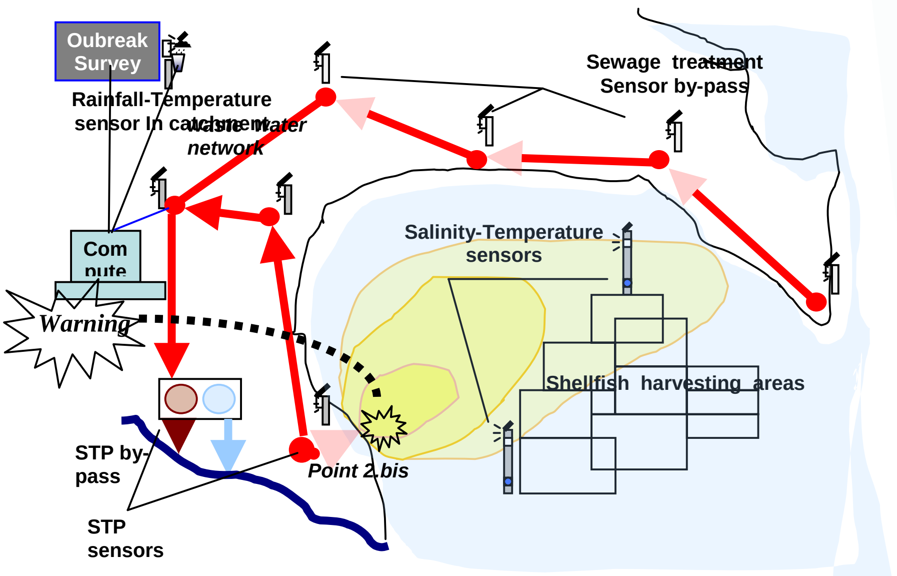

Figure 2 : Early warning system implementation and fonctionment

In situ validation of selected parameters is necessary for such system: comparison of resulting water quality conditions has to be established on the different sites and can serve as the basis for the quality water advisory. Recent development of such models based on simple relationship between the observed rainfall and pathogen concentrations or other based on complex modelling of the dominant mixing and transport processes are yet used for bathing risk managment (EAP, 1999). Thus water quality model could be applied and used to manage the shellfish risk. When validated, the early warning system would produce a model potentially usable at all European production sites.

Conclusion

Shellfish trade turnover represents 456 M Ecu per year in Europe: 775 000 t of shellfish are regularly travelling in EU countries to be consumed at different places. Free exchange of shellfish in the European market is important for sustainable development of shellfish production. Nevertheless the risk assessment must take into account the hazards compounded by the traditional consumption of raw or only lightly cooked bivalve shellfish. Shellfish and especially oysters are currently suspected to be involved in gastro-enteritis outbreaks occurring in different European countries. "Rapid Alert System for Foodstuffs" of the EU indicates, each year, shellfish from different origins are implicated in outbreaks in Europe. To limit this risk different propositions have be done including HACCP procedures, depuration (Jackson & Ogburn, 1997; NSSP, 1993; Furfari *et al.*, 1992; West, 1986). But due to the persistence of some species of viruses, viral risk still could exist even after a reinforced purification. For these reasons, further investigations in harvesting area are suggested to preserve water quality from microbial contamination, rather than to only focus on shellfish depuration.

Critical points in harvesting areas and « risk months » have to be taken into consideration to establish a warning system able to alarm shellfish producers and administration when a contamination event is potentially occurring. This warning system could avoid contaminated shellfish to be sold and thus consumer protection could be enhanced. When viral alert occurs, rigorous depuration has to be set up. Traceability and HACCP procedures have to be re-inforced to produce secure and safe shellfish. If applied, these recommendation will provide a real benefit for Europe, concerning the economic development (shellfish trade), social stability (employments), safety and health protection and Quality of life (reducing the contamination in coastal areas.

Acknowledgments: *This study was supported by Virus Safe Seafood grant (QLKI-1999-00634). We thank D. Hervio-Heath for valuable remarks on manuscript. We also thank Y. Madec (Ets Prat ar Com) for providing facilities for depuration experimentation. Thanks for S. Parnaudeau, C Le Mennec for the excellent technical assistance.*

References

Berg D.E., Kohn M.A, Farley T.A., McFarland L.M. (2000). Multistate outbreaks of acute gastroenteritis traced ti fecal-contaminated oysters harvested in Louisiana. *J. Infect. Dis.*, 181, a381-a386.

Bosch A., (1995), The survival of enteric viruses in the water environment. *Microbiologia SEM*, 11, 393-396.

Butt AA, Aldridge KE, Sanders CV, 2004, Infections related to the ingestion of seafood. Part 1: viral and bacterial infections. *Lancet Inf. Dis.* 4, 201-210;

Crowther J., Kay D., Wyer MD., 2001. Relationships between microbial water quality and environmental conditions in coastal recreational waters: the fylde coast, UK, *Wat. Res.* 35 : 4029-4038.

Crowther J,. Kay D., Wyer MD., 2002. Faecal-indicator concentrations in waters draining lowland pastoral catchments in UK: relations with land use and farming practices. *Wat. Res.* 36: 1725-1734.

Dérolez V., 2003. Méthode de caractérisation de la fragilité microbiologique des zones conchylicoles, rapport de stage , ENSP, 55 p.

Doré W.J. & Lees D.N., 1995. Behavior of *Escherichia coli* and male-specific bacteriophage in environmentally contaminated bivalve molluscs before and after depuration. *Appl. Environ. Microbiol.*, 61: 2830-2834.

Doré W.J.K., Henshilwood K,. Lees D.N., 2004. Viral clareance during depuration at elevated temperature determined by an index organism and direct quantification of Norovirus. *Water and Sciences Technology (in press)*.

Furfari S. A., Kelley-Reitz D. J. et al., 1992. Hazard analysis critical points (HACCPs) and verification studies at shellfish depuration plants in the USA. . "*Shellfish depuration*", 2d International Conference" edited by R. Poggi & J.Y. Le Gall, Ifremer, Plouzané. 185-197.

Gerba, C. P. (2000). Assessment of enteric pathogen shedding by bathers during recreational activity and its impact on water quality. *Quantitative microbiology, 2*, 55-68.

Jackson KL., Ogburn DM., 1997. Review of depuration and its role in shellfish quality assurance, (project N°96/355), *NSW Shellfish Quality Assurance Program,* series ISSN 1440- 544, 77.

Koopmans M. & Duizer E. (2004): Foodborne viruses: an emerging problem. *International Journal of Food Microbiology* **90**, 23-41.

Lees D. 2000. Viruses and bivalve shellfish. *Int. J. Food Microbiol.* 59: 81-116.

NSSP, 1993. Manual of operations, US FDA, Prime Connection, http://www.fda.gov.

Miossec L., Le Guyader F., Haugarreau L., Comps M.A., Pommepuy M., 1998. Possible relation between a winter epidemic of gastroenteritis in France and viral contamination of shellfish. *J. Shellfish Res.*, 17: 1661-1664.

Pommepuy M., Dumas F., Caprais MP., Camus P., Le Mennec C., Parnaudeau S., Haugarreau L., Sarrette B., Vilagines P., Pothier P., Kholi E., Le Guyader F., 2003. Sewage impact on shellfish microbial contamination *Water and Sciences Technology (in press)*.

Pommepuy, M., Caprais, M.P., Le Saux, J.C., Le Mennec, C., Parnaudeau, S., Madec, Y., Monier, M., Brest, G., Le Guyader, F., 2003.Evaluation of viral shellfish depuration in a semi-professional size tank. *In Molluscan Shellfish Safety*. Villalba A, Reguera B., Romalde J. & Bieras R. (Eds.) 485-499.

Sobsey M.D. & Jackus L.A., 1991. Human enteric viruses and depuration of bivalve mollusks. In: *Molluscan shellfish depuration*. W.S. Otwell, G.E.Rodrigues & R.E. Martin, Edts. Boca Raton, Florida: CRC Press Inc. 71-142.

Wait D.A. and Sobsey M.D., 2001. Comparative survival of enteric viruses and bacteria in Atlantic Ocean Seawater. *Wat. Sci. Tech.* 43: 139- 42.

West P.A., 1986. Hazard Analysis Critical Control Point (HACCP) concept: application to bivalve shellfish purification. *J.R.S.H.* 4: 133- 40