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Shellfish contamination by norovirus : strain selection based on ligand expression?

Françoise S. Le Guyader[a], Robert L. Atmar[b], Haifa Maalouf[a], Jacques Le Pendu[c]

[a] Laboratoire de Microbiologie, IFREMER, BP 21105, 44311 Nantes cedex 03, France.
[b] Department of Medicine, Baylor College of Medicine, 1 Baylor Plaza, MS BCM 280, Houston, TX 77030, USA.
[c] INSERM U892, CNRS, 6299 ; Institut de Recherche Thérapeutique de l'Université de Nantes, 8 quai Moncousu, BP 70721, 44007 Nante cedex 1, France.

*: Corresponding author : Françoise S. Le Guyader, tel. : 33 240374052 ; fax : 33 240374027
email address : sleguyade@ifremer.fr
ratmar@bcm.edu ; jlependu@nantes.inserm.fr

Abstract:

Shellfish can be a vector for human pathogens. Despite regulation based on enteric bacteria, shellfish are still implicated in viral outbreaks. Oysters are the most common shellfish associated with outbreaks, and noroviruses, which cause acute gastroenteritis, are the most frequently identified pathogen in these outbreaks. Analysis of shellfish-related outbreak data worldwide shows an unexpected high proportion of NoV GI strains. Recent studies performed *in vitro*, *in vivo* and in the environment indicate that oysters are not just a passive filter, but can selectively accumulate norovirus strains based on virus carbohydrate ligands shared with humans. These observations may help explain the GI/GII bias observed in shellfish-related outbreaks compared to other outbreaks.

Bivalve molluscan shellfish, such as oysters, can filter large volumes of water as part of their feeding activities and are able to accumulate and concentrate different types of pathogens from fecal human pollution. When in shellfish, bacteria and viruses show differences in terms of concentration, accumulation and depuration [1]. As a consequence, absence of virus contamination cannot reliably be deduced from failure to detect bacterial contamination. Among human enteric viruses, noroviruses (NoVs) are recognized as being the leading cause of epidemics or sporadic cases of gastroenteritis in all age groups of humans [2, 3], and are also the leading cause of foodborne illness in the United States. Shellfish represent 13% of cases among outbreaks with a single food implicated, after leafy vegetables (33%) and fruits/nuts (16%)[4]. The likely point of contamination for all the mollusk-associated outbreaks in which this could be determined occurred during production or processing [4]. Considering that processing strategies to inactivate these viruses in shellfish are negligible [5], it is important to prevent contamination. Improved understanding of NoV behavior in shellfish may lead to increased sanitary quality of shellfish on the market.

1. Norovirus

NoVs belong to the *Caliciviridae* family, a group of non-enveloped, icosahedral viruses with a single-stranded, positive sense, RNA genome [3]. These viruses are highly diverse and are currently divided into 5 genogroups [6]. Genogroups I (GI), II (GII) and IV contain human strains, and currently the human strains cannot be cultured in vitro. Each genogroup is further subdivided into genotypes based upon analyses of the amino acid sequence of the major capsid protein, VP1. Other genotyping systems based upon shorter sequences [7] or analysis of the polymerase gene [8] have also been described. Additional strains and genogroups infecting animals have been characterized [6, 8]. NoV infection causes gastroenteritis that is characterized by vomiting and diarrhoea [3]. The prevalence of vomiting along with the short incubation period (1-2 days) and short clinical illness (1-3 days) have been used epidemiologically to identify probable outbreaks of NoV-associated gastroenteritis [3]. The infectious dose 50% has been estimated to be as low as fewer than 18 virions [9]. NoVs bind to histo-blood group antigens (HBGAs), phylogenetically highly-conserved, complex glycans present on many different cell types and proposed to be an attachment factor necessary to initiate infection in people [10, 11].

NoVs are the major cause of epidemic non bacterial gastroenteritis worldwide and have been identified as the cause of 73% to more than 95% of outbreaks [3]. These outbreaks involve all age groups in a wide variety of settings, with a large dominance of GII strains that can constitute up to 90% of clinical strains [2]. Over the past 10 years, NoV sequence analyses of outbreak strains collected from around the world show that GII.4 viruses have accounted for ~70% of all human cases [12].

2. Coastal area contamination.

The regular and predictable pattern of seasonal outbreaks dominates the epidemiology of many exclusively human pathogens [13]. For NoVs, a clear peak of outbreaks occurs during cold weather months on several continents, with less ultraviolet light, cold temperature, and frequent run-off being some of the possible explanations for extensive transmission [14]. However, NoVs continue to circulate endemically throughout the year, and although there is the theoretical possibility of zoonotic spread, currently there is no direct evidence of the existence of a reservoir for re-introduction into the human population [15]. As a consequence, it is now evident that some strains may be detected all year long, either in sporadic cases of illness or in untreated sewage [16, 17]. NoVs, being very resistant to

inactivation, may persist in outflow water after treatment in a sewage treatment plant. Indeed, they have been frequently detected in treated waters and surrounding rivers. Although wastewater is treated for the purposes of removing bacterial and viral pathogens, treatment is not 100% effective and wastewater effluent may contain enteric viruses that can contaminate the environment. Concentrations of hundred to thousands of genomic copies per liter of treated wastewater can be detected, and seasonal variability is similar to that seen for untreated sewage. Several studies have reported a higher frequency of GI NoV strains in treated effluent compared to GII strains [16, 18, 19]. GI NoVs are more resistant to inactivation and removal during the sewage treatment process, though the reason for this is unclear and needs to be studied. One hypothesis is that differences in capsid protein or binding properties may be responsible for the different treatment efficiencies observed amongst NoV genogroups [20]. New technology for sewage treatment such as the membrane bioreactor, which more efficiently eliminates all small particles including viruses, may be an effective alternative to conventional treatment and will avoid strain selection [21].

Limited data are available to calculate “virus based-flow” or “event-flow” discharges into rivers or estuaries. Nevertheless, published data suggest that, during non-epidemic periods, less than 10^3-10^4 genomic copies/liter of NoV are present in treated wastewaters, whereas, during the epidemic period (winter) the concentration is probably 100- to 1000-fold higher [18, 21, 22]. Viral elimination depends on a wide array of factors, including temperature, solar radiation, adsorption, enzymatic destruction, and predation by bacteria and protozoa. Removal mechanisms are complex and difficult to elucidate, especially for non-culturable viruses such as human NoVs. For example, the association of NoV with particles may either protect from inactivation through shielding of the particle-associated virus or enhance inactivation by photosensitization of adsorbed macromolecules as demonstrated for other viruses [20, 23, 24]. Nevertheless, these data indicate that viruses are discharged into environmental waters with a seasonal profile and raise questions about the frequency and duration of such peaks and the importance and impact of storm events that result in a bypass of wastewater treatment during high flow epidemic periods. In the absence of precise information, calculations from epidemiological data suggest that 10^6 NoV fluxes can be expected from a town of 60,000 population-equivalent during winter outbreaks [25]. Many environmental factors can have an impact on virus distribution, including currents, estuaries, and tides [25]. Despite efforts to reduce pollution, human activities produce wastes that are discharged into the sea. When entering in the sea, the free- or bound-microorganisms are subjected to dilution and bio-sedimentation processes.

3. Oyster contamination

Shellfish pump water over their gills, and suspended particles are captured and passed on to the alimentary tract. However, some sorting of particles occurs prior to ingestion to help regulate what is presented to the digestive tract. Food particles enter the stomach through the short esophagus, and particles are further sorted according to size, density and digestibility. The ciliary action of epithelial cells sorts the particles in the stomach as follows: small and heavy (or excess) particles are immediately rejected through the intestinal groove to the midgut while larger or lighter particles are recirculated for further degradation. Food is moved from the mouth toward the anus by the strong ciliary activity from epithelial cells that line the alimentary tract. The digestive gland surrounds the stomach entirely and also surrounds part of the intestine. It is comprised of a series of branched ducts that open into the stomach. Each duct branches serially to terminate in blind-ending tubules, the location of the digestion activity. Food particles are embedded in mucous strings from the esophagus and are carried forward by the rotation of the crystalline style and subjected to mechanical and chemical (mainly glucanases) degradation. Small particles and insoluble molecules enter the digestive gland via the brush-border of the ducts. A second phase of extracellular digestion occurs in the lumen of the tubules, where extracellular enzymes are present.

However, intracellular digestion is the main digestive process in this part of the alimentary tract. Nutrients are then transported to the hemolymph, amoebocytes and periglandular connective tissue. Undigested remnants accumulate in residual bodies. In the final phase of the digestive process, the digestive cells break up to release their apical pole filled with residual bodies and lysosomes, and these are expelled into the lumen of digestive tubules, thereafter reaching the stomach via the ciliated duct section. Waste products are passed on to the rectum via the intestine, where digestion and absorption of some nutrients may also occur [26, 27]. All organs involved in the ingestion and digestion of food including the mouth, a short esophagus, stomach, crystalline style sac, digestive diverticula, midgut, rectum and anus are usually called "digestive tissues". With the exception of a short section of the rectum, the entire alimentary canal lies within the visceral mass and is completely immobilized by the surrounding connective tissue (called mantle tissues).

4. NoV ligands in oysters.

It was generally thought that oysters act as mere filters or ionic traps, passively concentrating particles such as bacteria or virus. However, unlike enteric bacterial species, enteric viruses persist in shellfish for an extended period of time. It is this persistence that currently results in the significant impact of shellfish-associated viral disease on public health. Viruses are principally concentrated in digestive tissues. A number of different mechanisms have been suggested to explain differences in virus accumulation between different oyster species, including mechanical entrapment and ionic bonding [28-30]. Virus accumulation in oysters can also depend on factors such as water temperature, mucus production, glycogen content of the connective tissue, and gonadal development. The importance of secreted acid mucopolysaccharides in the concentration of poliovirus was first demonstrated 30 years ago [30]. If oysters acted as filters or ionic traps, passively concentrating particles, a simple depuration process should be sufficient to rid oysters of virus as observed for bacteria. However, this is not the case. For example, only 7% of Norwalk virus (the prototype NoV strain) is depurated compared to a 95% reduction level of bacteria [29].

Several years ago, we tested whether oysters can actively capture a NoV, and we examined the possibility of specific binding to oyster tissues through related carbohydrates. We demonstrated that Norwalk virus specific binds to the oyster digestive tract through an A-like carbohydrate structure indistinguishable from human blood group A antigen [31]. Subsequently, this observation was confirmed in different oyster species and for other NoV strains [32, 33]. Interestingly, characterization of a blood group A activity in the acidic polysaccharide fraction from *Craossostera gigas* viscera was reported quite a long time ago [34]. We confirmed that GI.1 VLPs bind mainly to digestive tissues but not to other organs, consistent with the results of bioaccumulation studies performed with Norwalk virus (GI.1) and RT-PCR detection [35, 36]. However, the genetic diversity of NoVs is also reflected in the diversity of their binding capacity to various human HBGAs structures [10, 11]. Differences observed between GI.1 and GII.4 binding to HBGAs were also present in oyster tissues [36]. Data demonstrated that the distribution of GII.4 is not restricted to digestive tissues as observed for GI.1, in accordance with reports demonstrating the presence of GII.4 NoV in gills, albeit to a lower extent than in digestive tissues [37-39]. Our quantitative analysis is consistent with a lower expression of GII.4 binding sites in gills as compared to the digestive tissues, although the difference was not statistically significant [36]. In addition, we demonstrated that the binding to gills and mantle tissue sections involves a sialic acid in α2,3 linkage, whereas in digestive tissues the interaction involves both the sialic acid and an A-like carbohydrate ligand [36]. Very little is known about sialic acids and their distribution in oyster tissues [40]. Some differential recognition of these ligands by GI and GII strains may lead to distinct outcomes in terms of the persistence of viral particles within the different organs. In other words, recognition of the sialylated ligand by GII strains may lead to a quicker degradation or release, whereas recognition of the A-like ligand results in virus

persistence. Following accidental contamination by sewage of a producing area a few years ago, we found that after one week the number of shellfish containing GII NoVs was higher than for GI strains, whereas after three weeks the converse was true [41]. Although this hypothesis remains somewhat speculative and requires further evaluation, it may partly explain why GII strains that are shed in the environment in far larger amounts than GI strains are relatively less frequent causes of oyster-related outbreaks [7, 41-44].

For easier comparison between samples and NoV strains we developed an ELISA test, based on the soluble glycans recovered after tissue homogenization [36]. When applied to samples collected monthly over an 18-month period, this test demonstrated that there is a seasonal variation for the ligand expression. Oysters in France were able to bind much more efficiently to GI.1 VLPs during the first five months of the year (January to May) as compared to the rest of the year (Figure 1). The differences observed here may be linked to the water temperature, lower during the end of winter and beginning of spring. The influence of water temperature is difficult to analyze as many other environmental parameters, such as oyster physiology, chlorophyll A concentration, and phytoplankton levels, may have an impact [45]. Interestingly, the end of winter/beginning of spring period corresponds to the highest concentration of NoVs in sewage and to a time when heavy rainfall is common, both of which may increase the risk of oyster contamination following failure of sewage treatment plants or during flooding [16, 46, 47]. Although present, the seasonal effect was markedly less apparent for GII.4 VLPs, suggesting that the corresponding strains may be accumulated with more or less similar efficiency all year round [39]. In France, the peak of oyster-related outbreaks occurs at the beginning of the year [41, 42, 48], whereas the peak of consumption lies between December 24-31 [49].

5. Impact of ligand on bioaccumulation.

In order to get a more complete picture of the strain-specific potential for contamination of oysters, viral bioaccumulation studies in oyster tissues using one representative GI and one GII strain were undertaken. *r*RT-PCR quantification of virus recovery from *in vitro* bioaccumulation experiments performed at several time points during the year was performed in parallel with measurements of ligand expression during the same periods. The impact of these ligands on NoV bioaccumulation in oysters, was compared for the two NoV strains in terms of efficiency of bioaccumulation, tissue distribution and seasonal influence. Selection of tissues analyzed was based on VLP binding ability but also on oyster physiology, as described above. Based on these parameters, we chose to apply a quantitative approach to three groups of tissues i.e. gills, digestive tissues and mantle.

The first striking observation was that oysters concentrated the two strains with different efficiencies and tissue distributions. The GI.1 strain was previously shown to bind specifically through an A-like carbohydrate structure to DT but not to other tissues. We observed that it was readily bioaccumulated in DT with less than 1% of the virus detected in other tissues after 1 hour and a 1000-fold difference compared to the gills/mantle after 24 hours, consistent with the lack of a ligand in gills and mantle (Figure 2). The concentration detected in DT increased as the amount of virus seeded into seawater increased. The high concentration of GI.1 recovered in DT is also consistent with earlier observations [35]. The efficiency of this DT-specific bioaccumulation paralleled the season-dependent expression level of the carbohydrate ligand; strongly arguing in favor of its involvement in the bioaccumulation process (figure 3).

For these bioaccumulation experiments, a GII.3 strain was selected as it may be more resistant in sea-water than GII.4. Moreover GII.3 NoVs have a similar ELISA binding pattern to oyster tissues as that observed for the GII.4 strain, with VLPs binding to DT, gills and mantle. After one hour, NoV GII.3 was detected in gills and mantle but also in DT (Figure 2),

with the level of detection dependent on the concentration of virus seeded into seawater. After 24h, gills and mantle tissues displayed concentrations 1000-fold lower than in DT, suggesting that after being transiently retained in the gills, probably due to binding to sialic acid, they are either destroyed, or they are released to enter the mouth as observed for the GI.1 strain. The release from the gills or mantle might occur if the GII.3 strain has a lower binding affinity for the sialic acid-containing ligand, an aspect that will require further investigation.

Shellfish species may also impact bioaccumulation as demonstrated in a comparison of two oysters species (*Crassostrea ariakensis* and *C. virginica*). The GI.1 strain was more efficiently concentrated by *C. ariakensis* and persisted for a longer time compared to *C. virginica* [50]. It will be interesting to compare the glycan ligand expression between these species.

6. Naturally contaminated oysters

Since many environmental conditions can interfere with an oyster's filtering capacity and consequently with contamination, a field study was conducted to determine if the above observations performed under laboratory conditions are valid in the environment. In France shellfish are grown in coastal waters. Such shellfish can be exposed to human fecal contamination, but they also can be exposed to fecal contamination from cattle in neighboring fields. As a consequence, the shellfish can be contaminated by the animal strains, raising an issue of the potential role of oysters in the emergence of bovine NoVs into the human population. A study was conducted to provide quantitative data on the presence of GIII NoV in the environment in comparison to GI and GII strains and to evaluate the ability of GIII NoV to bind to shellfish tissues [51]. Tracing viral contamination in the open environment is quite difficult due to several factors such as source identification, dilution, currents and finally sampling strategy [25]. An area representing a bovine population at least an hundred fold greater than the human population in terms of sewage input (10^6 inhabitants equivalents for bovine compared with 10^4 inhabitants equivalents for humans) was selected, and the presence of NoV GIII in cattle was verified (18% of bovine stools were detected positive). Bovine NoVs were detected in a total of 14% water samples, and GI and GII NoVs were detected in 7% and 24% of water samples, respectively (Table 1). Moreover the sum of GI RNA copies detected in water during the whole study period was 28 times lower than that of GII, in accordance with epidemiological data that shows a large predominance of GII strains in the human population [3]. Considering NoV quantification obtained in oyster tissues, GI NoVs were detected less frequently than GII NoV but the sum of RNA copies was greater. If we calculate the ratio of viral RNA copies/L water to the viral RNA copies/g oyster tissues, NoV GI were concentrated to a greater degree than GII strains, with the ratio being 30 for GI compared to 1 171 for GII strains. These data provide additional evidence for the specific selection and persistence of GI NoVs in oysters. The sum of GIII RNA copies detected was only twice lower than that of GII human NoVs, showing the high impact of bovine production on water contamination. In Brittany, bovines are bred in open fields most of the year and thus feces may directly contaminate small nearby rivers. Considering the substantial percentage of positive water samples and the long persistence of human NoVs in contaminated oysters, we were surprised to find only one shellfish sample positive for GIII NoV. Because extraction and inhibitor controls were used, and primers and probe set used readily detected GIII viruses in bovine stools, we think that negative samples were truly negative. However, the αGal HBGA epitope, identified as the virus-specific glycan ligand in bovine tissues [52], was absent from oyster tissues, potentially explaining the poor bioaccumulation efficiency observed for GIII NoV strains. Nevertheless, a weak binding of both NB2 and BEC28 VLPs to unidentified structures of oyster digestive epithelial cells was detectable, consistent with the rare and quantitatively weak detection of GIII sequences in oyster samples compared with the frequent water contamination. In bioaccumulation

experiments no GIII NoV VLPs were detected in oyster tissues, suggesting that the lack of specific ligand in these tissues may help to explain the rare occurrence of GIII oyster contamination despite frequent water contamination. In contrast, GI VLPs, which have a specific carbohydrate ligand in oyster digestive tract, were efficiently bioaccumulated as previously described [36]. This is in accordance with the low ratio of GI RNA copies between water and oyster samples found in our environmental study. Alternatively, we cannot exclude the possibility that GIII strains, as well as GII strains, may not accumulate and persist in oysters as efficiently as GI strains because of a lower stability. Thus, GI NoVs could accumulate and persist in oysters because of high environmental stability in addition to the presence of a specific ligand. In contrast, GII and GIII strains would accumulate and persist for a shorter time because of the absence of one of these two characteristics.

7. Shellfish-related NoV outbreaks

Shellfish have been known to be a high-risk food for viral outbreaks for many years, but clear strain identification in shellfish is still often difficult. One of the first reports providing the sequence of a NoV strain described an outbreak in the US. A GI.4 strain was found in oyster samples, but the sequence was not identical to those detected in patients' stools [53]. At the same time in Japan, a mixture of GI and GII NoVs was detected both in stool and the related oyster samples but no sequencing was performed [54]. Since then, improvements in detection methods and the development and harmonization of molecular typing strategies have simplified data comparisons, allowing a compilation of outbreak reports that used comparable methods (Table 2).

One characteristic of shellfish-related outbreaks is their frequent association with multiple virus strains observed both in infected patients and in the involved shellfish. This likely reflects contamination of shellfish waters with human sewage where multiple genotypes are present rather than fecal contamination from a single NoV-infected individual. When a number of different virus strains are detected in patients, association of the infection with shellfish consumption can be difficult if only a few stools from an outbreak are collected. Thus, it is essential to collect as many stool samples as possible from affected individuals so that all strains that may be present can be identified. It is also important to rapidly identify the outbreak in order to trace the oyster production and to quickly collect the samples related to the outbreak. These data can be used with collected epidemiological data to fully understand the role played by shellfish in the outbreak.

Primers and probe sets specific for each NoV genogroup have been developed for detection by real time RT-PCR [55]. However, genotyping remains a challenge, especially in shellfish where low viral concentrations are observed and in stools containing several different strains. In addition, a cocktail of primers is often required to detect the various NoV strains because of the diversity of these viruses [6, 8].

Most outbreaks of shellfish-associated NoV disease are linked to oyster consumption, presumably because oysters are the most commonly consumed shellfish and they are usually consumed raw (although some outbreaks have been linked to cooked oysters) [56]. Overall, contamination by multiple NoV strains has been reported in 65% of reported outbreaks, with GI and GII NoVs detected, respectively, in 71% and 88% of stool samples and in 75% and 92% of shellfish samples. The frequency of each genogroup detected in shellfish-related outbreaks is clearly distinct from that of other NoV outbreaks. GI strains are more frequently encountered in shellfish-related outbreaks, and the GII.4 genotype is not as dominant (Table 2). Among GI NoVs, the most frequently reported genotype is GI.1, followed by GI.4 and GI.2. Among GII NoVs, the GII.4 genotype is the most frequently reported from both stool and shellfish samples, but was reported as frequently as the GI.1 strain [57].

Some reports provide only stool analyses without shellfish data, such as the description of GI.1 and GII.3 strains implicated in an oyster-related outbreak reported from the UK [44]. In Japan GI NoVs alone were detected in four out of 11 outbreaks related to oyster consumption, with the remaining 7 outbreaks being associated with a mixture of GI and GII NoVs. In that study, GI.1 strain was detected in 3 of the 11 eleven outbreaks [58]. A previous study, also from Japan, reported the presence of a mixture of GI and GII NoVs in stools from 19 out of 21 oyster-outbreaks. In contrast, of 45 outbreaks not linked to shellfish consumption, all but 3 were due to GII NoVs, with both GI and GII strains being found in the remaining three [7].

8. Conclusion

These data suggest a selective transmission of NoV strains via oysters through specific binding to carbohydrate ligands. Ligands that facilitate bioaccumulation (the A-like antigen) or that contribute to the elimination of the virus (the sialic acid-containing ligand) may both influence NoV accumulation and survival in oysters. A new approach combining *in vitro* studies and environmental samples analysis brings valuable information to investigate the possible contamination of shellfish.

For a long time, oysters were believed to act simply as filters or ionic traps, passively concentrating particles. However, this is clearly not the case for NoVs, especially for NoV GI.1 that is more actively and efficiently concentrated than GII strains. The differential accumulation efficiency provides a possible explanation for the unexpectedly high proportion of GI strains associated with shellfish-related outbreaks.

This new concept demonstrating a special relationship between oysters and NoV should be explored for other enteric viruses and other shellfish species. It will be important also to evaluate different countries of production as environmental conditions have a clear impact on ligand expression. As food trade may contribute to the occurrence of large outbreaks and widespread virus distribution, a better understanding of virus-food interactions may provide strategies to prevent contamination, to increase viral elimination, and thus to improve consumer safety.

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Tables

Table 1. Quantification of NoVs GI, GII and GIII in environmental samples during a one year filed study (adapted from [51]).

NoV	Water samples (Nb=70)[a]		Oyster samples (N=47)[a]		
	Nb. of positive samples	Sum of NoV RNA copies/liter	Nb of positive samples	Sum of NoV RNA copies/ g of DT	Water/ Oysters[b]
GI	5	11,510	2	381	30
GII	17	325,530	10	278	1,171
GIII	10	142,220	1	90	1,580

[a]Total number (Nb) of samples
[b]Ratio of the sum of RNA copies detected in water and oyster samples during the study

Table 2. Norovirus genotypes detected in shellfish related outbreaks (adapted from[57])

year	Country	Stool samples		Shellfish samples		Reference
		NoV GI	NoV GII	NoV GI	NoV GII	
2000	France	1, 2, 3		1		[59]
2001	Netherlands	1, 4	b, 7	4	7	[60]
1998-2002	Japan[a]	1-5, 7-9, 11-14	1, 3-12, 14, 16	No sample		[7]
2002	Italy	4	8, b	4	b	[61]
2002	France	4, 6	4, 8, b	4	4, 8	[42]
2003-2004	Australia[b]	2, 4	5, 6, 7, 9, 12		4	[62]
2004	UK	1, 2	3, 4	No sample		[44]
2004	Canada	1, 2	3, 4, 5,	1	12	[63]
2005	Japan		1, 4, 5, 6	No sample		[64]
2006	New Zealand		3, 6, 12	3	3, 6, 8, 12	[65]
2006	France	1, 2, 4	2, 4, 7, 17, b	1, 2, 4	4, 17	[41]
2002-2007	Japan[c]	1-5, 8, 10, 13-15	3-6, 8, 12	No sample		[58]
2007	Sweden	1		1	3	[43]
2008	France		4		4	[48]
2008	Japan	1	4, 8	1	8	[66]
2009	US		12	?	?	[56]

Data presented in some papers represent 21(a), 14 (b), 38 (c) outbreaks.

Figures

Figure 1 : Seasonal variation of VLP binding to oyster digestive tissues extracts.
The binding of GI.1 (black bars) and GII.4 (grey bars) VLPs to DT extracts form collected oysters over 1 year (x axis), expressed as the signal/noise ratio ± SD (y axis) was determined. The GI.1 binding during the period from January to May is significantly different from the binding during the period from June to December (dotted line) (p= 4.8 x 10^{-12}) (adapted from [36]).

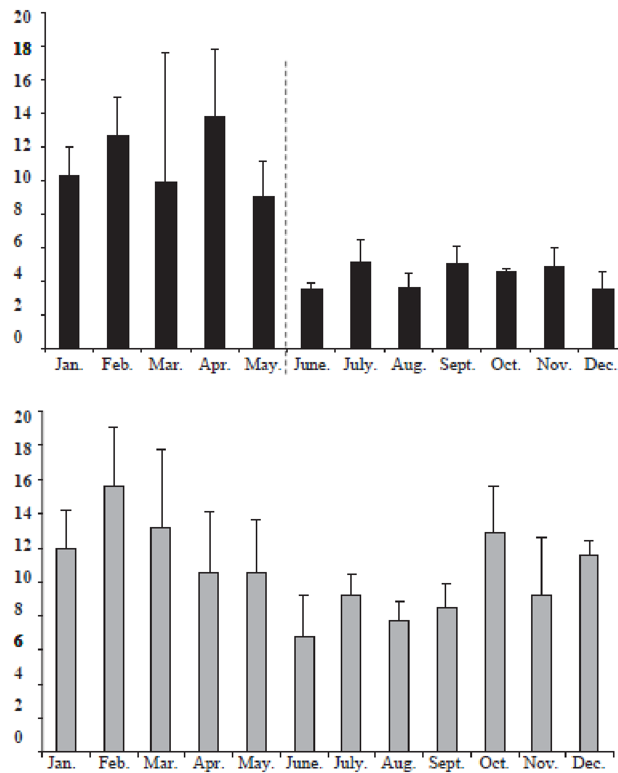

Figure 2 : GI.1 and GII.3 bioaccumulation in oysters.

Concentrations in DT at 24 h are reported as genome copies (y axis) for the four experiments (October, November, January and March) (x axis) for GI.1 (circle), and GII.3 (square). Three concentrations were assayed for the GI.1 bioaccumulation : A: 6.5 ±0.2 log10 RNA copies/liter, B: 10-fold higher concentration and C: 100-fold higher concentration and GII.3 bioaccumulation : A: 6.4 ±0.3 log10 RNA copies/liter, B: 10-fold higher concentration and C: 100-fold higher concentration (adapted from [39]).

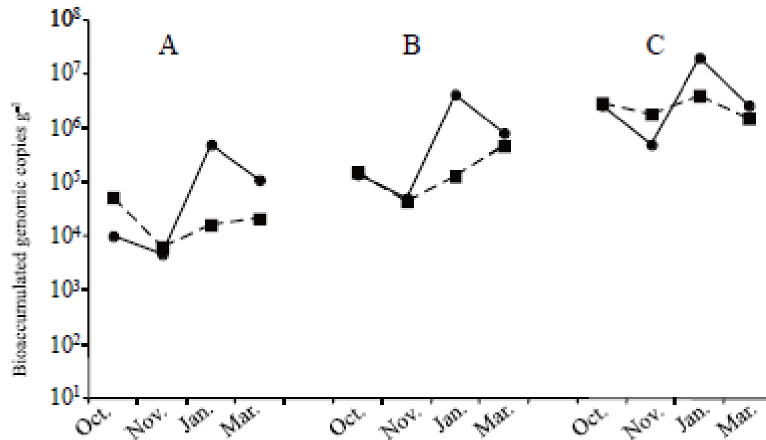

Figure 3 : Ligand expressions measured by VLP binding.

Binding of GI.1 (black bars) and GII.3 (grey bars) VLPs to extracts of DT for the four months (x axis) expressed as signal/noise ratio (y axis) (adapted from [39]).