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Evidence of the presence of viral contamination in shellfish after short rainfall events

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Abstract:

Infectious diseases linked to the consumption of raw shellfish have long been identified. Over the past century, various strategies have been set up in shellfish growing areas throughout the world to guarantee the sanitary quality of shellfish and to protect consumers. However despite sanitary improvements, human enteric viruses - especially Hepatitis A virus and norovirus– have been found to be associated with shellfish outbreaks. A recent example demonstrated the impact of storm events. Following heavy rain and sewage overflow, shellfish beds were contaminated and the shellfish from them were marketed after depuration. However, since viruses persist longer than fecal contamination indicator bacteria, several clusters of gastroenteritis cases were reported. Analysis of both clinical and shellfish samples showed the presence of up to eight different strains of viruses: norovirus genogroup I genogroup II, astrovirus, and rotavirus, all strains being identical between clinical and environmental samples. Finding such a mixture is an indication of a fecally contaminated source such as sewage, especially in winter when a high diversity of strains may be present. In the future, standardization of methods, a better understanding of virus epidemiology will lead to improved shellfish safety for the consumer and to protect shellfish growing areas and to identify all possible sources of contamination.

Keywords: virus, shellfish, contamination events

1. Introduction

Seashores are areas with large populations, attracted by the quality of life and the wide range of activities, including recreational activities, found there. A significant portion of the resulting human wastewater fluxes carrying pathogenic microorganisms find their way into coastal waters. Even though wastewater is usually treated before being released into streams and rivers, during heavy rain events, the pollution eventually reaches the sea from surface runoff or sewage overflow. Shellfish, and especially the filter-feeding bivalves that are normally eaten raw, are a commonly implicated vehicle for the transmission of infectious diseases. Even when harvested in good quality water, they may be subject to intermittent contamination leading to an accumulation of pathogens and a possibly significant health risk. From February to March 2007, outbreaks linked to shellfish consumption were reported to the Health Ministry in France. A rapid investigation concluded that oysters grown in a lagoon located in southern France were responsible. Currently, in this area classified B under an EU regulation (91/271/EEC), shellfish undergo depuration in tanks before being marketed. Shellfish from this area are mainly sold on local markets (74%), with the domestic market accounting for 24% and the European market 2%. This study reports the investigations and results obtained during this period by scientific and administrative partners in order to understand the source of the contamination and manage the risk.

2. Material and methods

Environmental investigation

The oysters (*Crassostrea gigas*) used were collected from January to March 2006. Samples were taken from three levels of sources, one involved oysters from a family cluster (home refrigerator), related shellfish from the same producer (shellfish from the same bag as those implicated) and from the harvesting area, more particularly from the beds that the oysters consumed came from. The oyster flats in question are located in a lagoon in the south of France where a surface area of 2,142 ha is devoted to shellfish production. Shellfish are grown on 2,750 long-lines, with about 13,000 T of oysters (*Crassostrea gigas*) and 2,500 T of mussels (*Mytilus edulis*) are produced yearly. Environmental data covering the period from January to March, 2006, were collected from local authorities and services. They included data on rainfall (Météo-France), sewage treatment plant efficiency (sewage treatment plant operator), river flow (Languedoc-Roussillon DDE regional infrastructure service's lagoon monitoring network) and the population's epidemiological status (Sentinelle network: http://rhone.b3e.jussieu.fr/senti/). For the same period, information enabling shellfish traceability was obtained from the services of the Ministry of Health and of the Ministry of Agriculture. Finally, data on shellfish quality were collected from shellfish farmers and Ifremer's monitoring network (REMI:http://www.ifremer.fr).

Epidemiological data

On 7 February 2006, the first cluster of cases of acute gastro-enteritis was reported to the French public health authority. From this date up to 27 February 2006, a total of 37 outbreaks occurred in southern France. A cohort study was set up and for all clusters of cases, a standardized questionnaire covering all food consumed and the symptoms and timing of the illness was completed for each participant to identify possible sources of contamination. A link between shellfish consumption and the illness was rapidly established (85%), orienting the investigation towards collection of shellfish and stools. The results from four of these clusters of cases (A, B, C and D) are reported here because complete information was available for them in order to compare stool and shellfish analyses. Cluster A occurred during a scientific workshop where 28 people fell ill (3 February), group B on 4 February from a family meal (2 people sick), and C and D, with 12 cases, were traced back to two family meals, featuring shellfish bought at the same local market on 4 and 11 February. Twelve stool samples were collected from these 4 clusters and analysed.

Clinical sample analysis

Before analysis, the RNA was extracted using Qiamp viral RNA kits. The same primers used for the shellfish samples were used to detect enteric viruses (Bon et al. 2004; Le Guyader et al. 2006).

Shellfish sample analysis

Shellfish kept at 4°C during shipment were washed and shucked. The stomachs and digestive diverticulae were removed by dissection, divided into 1.5-g samples and then analyzed as previously described (Le Guyader et al. 2006). Noroviruses (genogroups I and II), enteroviruses, rotaviruses and astroviruses were detected by conventional RT-PCR as well as real-time RT-PCR (for norovirus) (Le Guyader et al. 2006, Loisy et al 2005) *E. coli* counts were performed using the ISO 93038-3 technique in water and the NF-V-08-106 impedancemetry method in shellfish.

3. Results and discussion

The first suspected shellfish outbreak occurred on 2 February and was reported to the administration on 7 February. Shortly beforehand, following torrential rains from 27-29 January, a warning was issued by the enforcement authority, informing the administration in charge of shellfish quality and the shellfish producers. In fact, following the storm event, preventive analyses performed by Ifremer on 30 January, indicated concentrations reaching 4 800 *E. coli*/100 g in shellfish in some locations of the lagoon (REMI Ifremer network). Shellfish farmers were asked by the administration to increase the depuration time to 15 days. Nevertheless, despite the warning requiring additional depuration, a few days later other outbreaks presumably due to shellfish were already being reported. These reports led the administration to take shellfish off the market and then to close the shellfish flats for several weeks (figure 1).

Environmental investigations carried out in concurrently with the epidemiological investigation aimed to gather all the information which could help in understanding the origin and the events leading to the contamination of the shellfish (Faillie J.L, 2007). The Météo-France weather reports indicated that 150 mm of rain fell in the lagoon area over the 3 days from 27-29 January, with peaks of over 70 mm/day, and that 250 mm fell on the catchment. This unusual amount of rain increased the runoff, carrying fecal contamination into the sea. In addition, wastewater facilities and septic systems were also overwhelmed by the rainfall, since drain fields in saturated soils were less efficient. Likewise, heavy rain contributed to decreasing residence time in wastewater treatment ponds (nested ponds are used in this area) and thus the water released into the bay was insufficiently treated (SDEI operator, pers. com.). During the same period, intermittent raw water overflows from small tributaries were observed (SDEI operator, technical notes, 2006). Analyses performed in streams showed *E. coli* concentrations varying from 30,000 to 79,000 *E.coli*/100 ml (DDE data). The shellfish beds are located close (about 500 m) to the mouths of streams and were rapidly impacted by this poor quality fresh water input.

During the same period, over several weeks, a high number of gastroenteritis cases were reported in the French population (Sentiweb). This sort of large outbreaks of acute gastro enteritis occurs every year for 4-6 weeks in winter in the European population. These wintertime gastroenteritis events are mainly caused by noroviruses transmitted by direct contacts with people with diarrhea (Atmar & Estes, 2006.). In January 2006, the number of cases in the population was about 500 per 100,000 inhabitants in the south of France, and peaked at 700 per 100,000 inhabitants on January 15th. Chan et al reported that infected individuals can shed millions of virus particles in their feces. Consequently there are many types of viruses present in large numbers in sewage and streams which are potentially contaminated by feces. They can overflow into the sea, especially when sewage treatment processes fail to remove viruses (Myrmel et al., 2006; Ottoson et al., 2006; Rose et al., 1996).

The storm occurring at the end of January was responsible for a strong viral input into the lagoon, due to an unusual co-occurrence of the two phenomena of heavy rainfall and viral input from illness population in the catchment (Fig. 2). Extremely heavy rainfall preceded by drought, had already been implicated as a risk factor for seafood-borne disease (Lipp et al, 2001; Le Guyader et al, 2000; Miossec et al, 1998). REMI monitoring network results indicated a rapid increase of *E. coli* concentrations in shellfish immediately after these events.

Viral analyses performed on shellfish in February at REMI sampling stations (Table 1, c: shellfish from same area as those implicated in outbreaks) demonstrated the presence of several viral strains in shellfish, although the *E. coli* concentration was under the regulatory limits (<230 *E.coli*/100g). All the shellfish tested positive for astrovirus (11/11) and 5 of them for norovirus. The presence of multi-viral contamination of shellfish had already been observed in this area (Le Guyader, 2006) and was suspected of being related to contamination from sewage. In the shellfish implicated in or related to the outbreak (Table 1), multiple strain contamination was observed, involving astroviruses and noroviruses. Moreover, two rotaviruses were found in the implicated shellfish and one enterovirus in shellfish directly related to the outbreak (samples from home refrigerator).
Out of 12 stool samples from 4 clusters, no virus was detected in one sample, one sample tested positive for at least one virus, and two samples showed multiple contamination with astrovirus, norovirus gI and gII, and rotavirus. Analysis of the PCR results showed the presence of the same sequence for some norovirus strains (genogroups I and II) and astrovirus.

Conditions of shellfish contamination

The lagoon where shellfish are farmed is a complex system which is subjected to the influence of diffuse urban runoff, a highly urbanized area and an active shipping port. The lagoon's watershed is drained by only one permanent river and eleven temporary tributaries which are evenly distributed along the northern coast. Temporary tributaries are located less than 500m from shellfish farming zones, while the mouth of the permanent river is located more than 2,500 m from them. Data on freshwater inputs and fecal fluxes are available for the permanent river and for only one temporary river. The hydraulic function and amounts of bacteria discharged are still not known for the ten others. The lagoon's response to the fecal flow from these two rivers was studied by Fiandrino et al, 2003. When considering the main hydrodynamic features in the lagoon, both physical and biological processes play a role in the spatial extent and duration of water contamination. The interaction between hydrodynamic conditions (mainly wind effect) and river flows (including the temporal pattern) influences the spread of fecal plumes. Some authors have demonstrated that the distance between the river mouth and a shellfish breeding area was a critical factor. If this distance is large (which was the case of the permanent river here), only a floodwater input of bacterial discharge leading to a high concentration of bacteria in lagoon waters will generate a plume which can reach the shellfish flats. However, if the distance between the river mouth and the breeding area is short (the case of all the temporary rivers here), even a discharge of bacteria which is ten times lower (Loubersac et al., in press.) could lead to a significant contamination of shellfish.

During the January events, all the rivers of the watershed were in spate (DDE, pers. com.). In this context, it can be assumed that the shellfish farming area located near the mouths of tributaries was strongly contaminated by all of these stream flows. Moreover, it has been reported that oysters accumulate microorganisms in a very short time (Plusquellec et al, 1990) and that once contaminated, because of the high persistence of viruses in the environment shellfish will remain contaminated for several weeks (Loisy et al, 2005). During our investigation for this study, shellfish were found positive for norovirus for 6 weeks. These results showed that the biological and physical hydrodynamic conditions contributed to viral accumulation and persistence in shellfish.

4. Conclusion

The conclusion of the epidemic investigation, environmental study and viral analyses demonstrated the role of shellfish in the outbreaks. The information collected also highlighted the role of pathogens carried by streams from land and the conditions leading to shellfish contamination. At the end of January, all the environmental conditions which would increase viral input into the sea were present. Taking the lesson learned from this dramatic event in terms of risk management, we can conclude that warning indicators such as heavy rainfall, rivers in spate, wastewater network failure and epidemics present in the population could be very relevant in preventing risk. When these phenomena occur simultaneously and at high levels, administrative measures such as *E. coli* shellfish analyses must immediately be implemented. The results could rapidly indicate whether the contaminated plume has reached the shellfish beds. Positive *E. coli* results must be followed by viral analyses and monitoring during the following weeks because of the long persistence of viruses compared to *E. coli*. Immediate closure of the shellfish beds when all these indicators are positive could help limit the sale of contaminated shellfish on the market. Another major point learned from this study is that increased depuration time, even when fully complied with, does not protect consumers, because viruses are attached to shellfish tissues making it difficult to eliminate them during the depuration processes (Le Guyader et al 2006). Once aware of the system's vulnerability, following this event, all the partners involved in activities in this region (shellfish producers, sanitary services, scientists, regulators and the wastewater plant operator set up a project called “OMEGA” whose aims are recovery of water quality (diagnosing contamination sources on the watershed to limit inputs) and risk management (setting up warning systems).

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Table 1: Results obtained on stools and shellfish

	Number	**Av**	**Ev**	**Nov**	**Rv**
Stool sample	12	3	6	9	2
Shellfish					
a. implicated	2	2	0	2	2
b. related	6	6	1	4	1
c. same area	11	11	0	5	0

a. implicated: sample collected from home refrigerator
b. related: sample collected from same producer or same batch

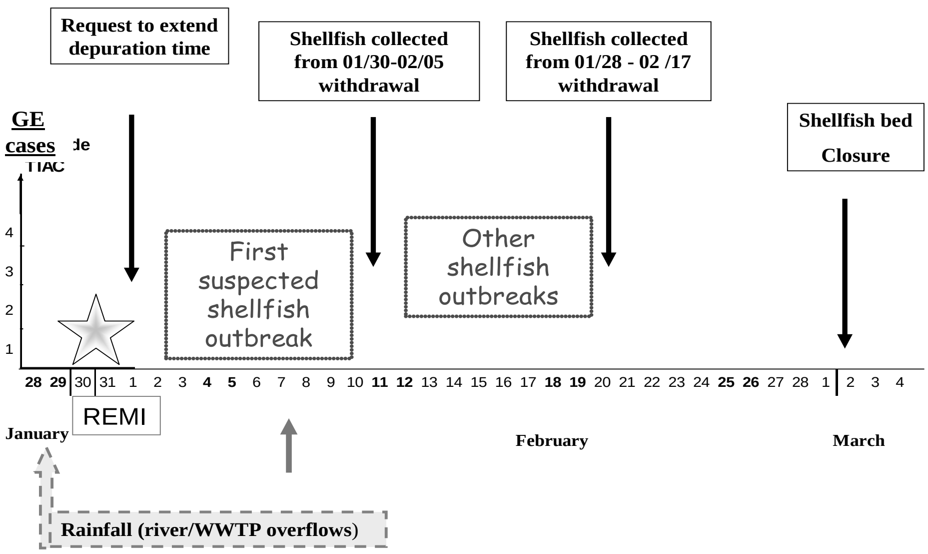

Figure 1. Events and decisions during the February-March period

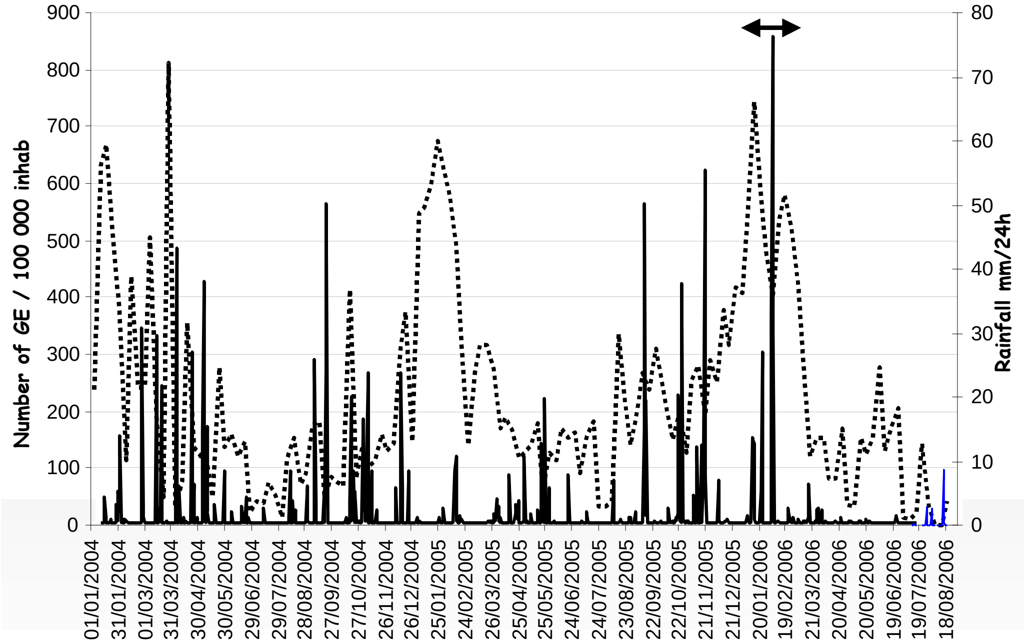

Figure 2: rainfall amount (**-**) and number of gastro-enteritis cases (**...**) in the population in southern France (Météo-France and Health Ministry survey) http://rhone.b3e.jussieu.fr/senti/).