The Director General

Maisons-Alfort, 10 February 2011

# OPINION
## of the French Agency for Food, Environmental and Occupational Health & Safety

**on a risk assessment relating to the reopening of a shellfish growing area closed due to the presence of calicivirus (norovirus and sapovirus) in live shellfish**

## 1. REVIEW OF THE REQUEST

On Wednesday 2 February 2011 the French Agency for Food, Environmental and Occupational Health & Safety received a request from the Directorate General for Food (DGAL) for an opinion on a risk assessment relating to the reopening of a shellfish growing area closed due to the presence of calicivirus (norovirus and sapovirus) in live shellfish.

## 2. BACKGROUND

**The following contextual points were mentioned in the DGAL's request:**

### 1. Context of the administrative decision to close the Étang de Thau

Following the appearance of different clusters of foodborne outbreaks (TIAC[1]), reported after the Christmas 2010 festivities and related to consumption of oysters from the Étang de Thau, the Prefect of the Hérault *département* issued a prohibition order on 6 January 2011 banning the harvesting and sale of shellfish from the Étang de Thau. This decision followed:

- the notification of seven clusters of TIAC involving 63 patients,
- results being obtained that demonstrated beyond doubt the presence of norovirus in oysters implicated in these TIAC,
- an unfavourable result being obtained from a microbiological analysis at one of ten microbiological surveillance network (REMI) points, with 7200 *Escherichia coli*/100 g flesh and intervalvular liquid, whereas the results should be below 4600 *E. coli*/100 g flesh and intervalvular liquid (classified area B) in accordance with the regulations in force (Rt 854/2004).

### 2. Establishment of a surveillance scheme based on screening for norovirus in oysters from the Étang de Thau

[1] TIAC: toxi-infection alimentaire collective, denomination used by the French Institute for Public Health Surveillance (InVS)

Following this TIAC episode, the National Reference Laboratory for Shellfish Microbiology at IFREMER[2] was asked to conduct weekly screening for the virus in shellfish samples taken at REMI points in the Étang de Thau. The results obtained on 3, 10, 17 and 24 January showed that the oysters found at most of the REMI points in the Étang de Thau were contaminated with norovirus and sapovirus, which substantiates the decision to close the area.

However, the results vary from one week to another. Some points which were negative one week could become positive the following week, or vice versa.

### 3. Other similar episodes dealt with in 2010

Closures were issued for two areas in Brittany last year based on the same type of results and contexts, in application of the provisions of Regulations (EC) No. 178/2002 and No. 854/2004. These areas remained closed respectively for four and six weeks until the disappearance of the viral signal at the surveillance points.

However, in areas that are known to be contaminated by viruses (such as the Bay of Paimpol, which is currently undergoing specific HAV and norovirus surveillance), no TIAC have been reported. These areas have therefore not been closed. This could indicate that there may be either a level of viral particles in shellfish below which the risk is very low, or a difference in pathogenicity between viral strains.

**Questions raised in the request:**

*"Are the viral risk and the viruses' level of pathogenicity as high, several weeks after the closure of the area, as at the time of its closure further to the TIACs?*

- *What objective and relevant evidence can the competent authorities rely on to consider that shellfish from this area is safe for human consumption?*
    - ✓ *signals from RT-PCR screening, provided by IFREMER as diagnostic evidence; a low number of viral particle copies demonstrating that shellfish contamination is low enough to authorise reopening?*
    - ✓ *an assessment of concomitant factors: weather, water temperature, etc.?*
    - ✓ *taking into account favourable results at a majority of REMI points, without waiting for a return to normal at 100% of the points?*
    - ✓ *other criteria…*"

## 3. EXPERT APPRAISAL METHOD

The collective expert appraisal was conducted by the "Norovirus-sapovirus/shellfish - Étang de Thau" Emergency Collective Expert Appraisal Group (GECU), which met by telephone conference call on 4 and 8 February 2011.

**It was conducted on the basis of:**

- The following information provided by the DGAL:
- Order no. 2011/011/045 of 6 January 2011 prohibiting fishing, gathering, transporting, purification, shipping, storage, and marketing for human consumption of non-burrowing bivalve shellfish - group 3 - from all areas of the Étang de Thau (Areas 34-38, 34-39 and 34-40)
- REMI alert bulletin no. 11/05 from the Environment & Resources Laboratory, Languedoc-Roussillon (LER/LR), microbiological control network, lifting alert level 2, zone no. 34-39 – group 3, 25 January 2011

[2] French Research Institute for Exploration of the Sea

- IFREMER test report no. RE/MIC-LNR/11.01 relating to screening for Calicivirus in shellfish, 5 January 2011
- IFREMER test report no. RE/MIC-LNR/11.02 relating to screening for Calicivirus in shellfish, 5 January 2011
- IFREMER test report no. RE/MIC-LNR/11.04 relating to screening for Calicivirus in shellfish, 6 January 2011
- IFREMER test report no. RE/MIC-LNR/11.08 relating to screening for Calicivirus in shellfish, 11 January 2011
- IFREMER test report no. RE/EMP-LNR/11.09 relating to screening for Calicivirus in shellfish, 13 January 2011
- IFREMER test report no. RE/EMP-LNR/11.13 relating to screening for Calicivirus in shellfish, 20 January 2011
- IFREMER test report no. RE/EMP-LNR/11.17 relating to screening for Calicivirus in shellfish, 28 January 2011
- IFREMER test report no. RE/EMP-LNR/11.19 relating to screening for Calicivirus in shellfish, 2 February 2011
- Summary table of TIAC due to oysters, Étang de Thau, end of 2010
- Agency Opinions and Reports:
- AFSSA Report: Review of knowledge on viruses transmitted to humans via the oral route (June 2007), 446 pages. ISBN 978-2-11-095835-8[3]
- AFSSA Opinion of 21 March 2008 on the environmental surveillance scheme and the assessment of the risk related to the consumption of shellfish, especially in the situation at the Arcachon Basin. Transmission of the response to the request (Request no. 2006-SA-0254) [4]
- AFSSA Opinion of 23 July 2009 on a request for an assessment concerning the surveillance procedures to be established in areas used for shellfish growing and gathering, that are regularly or accidentally contaminated by the hepatitis A virus (HAV), with application to the specific situation in the Bay of Paimpol (Request no. 2009-SA-0044)[5]
- ANSES Report: Contamination of marine shellfish by the hepatitis A virus (September 2010), 89 pages[6]
- Additional data provided by the IFREMER LER/LR laboratory:
- General information on the Étang de Thau
- Information on the environmental conditions (catchment area population, hydrodynamics of the lagoon, area's vulnerability to microbiological contamination)

[3] *Bilan des connaissances relatives aux virus transmissibles à l'homme par voie orale (juin 2007)*
[4] *Avis de l'Afssa du 21 mars 2008 relatif au dispositif de surveillance du milieu et d'évaluation du risque lié à la consommation des coquillages, notamment dans la situation du bassin d'Arcachon Transmission de la réponse à la saisine*
[5] *Avis de l'Afssa du 23 juillet 2009 relatif à une demande d'évaluation concernant les modalités de surveillance à mettre en place dans des zones de conchyliculture et de pêche à pied, régulièrement ou accidentellement polluées par le virus de l'hépatite A avec application à la situation spécifiquement rencontrée dans la baie de Paimpol (saisine 2009-SA-0044)*
[6] *Contamination de coquillages marins par le virus de l'hépatite A (septembre 2010)*

## 4. DISCUSSION

The argument of the French Agency for Food, Environmental and Occupational Health & Safety is based on the opinion of the "Norovirus-sapovirus/shellfish - Étang de Thau" GECU, whose main points are presented below:

### 4.1. Information on the hazard

#### 4.1.1. Norovirus - Sapovirus

Noroviruses (NoV) are non-enveloped viruses whose genome consists of a single-stranded RNA of positive polarity. They belong to the *Caliciviridae* family, genus *Norovirus.* The genus *Norovirus* includes five genogroups, among which the genogroups (GG) I, II and IV are pathogenic to humans. Noroviruses are characterised by great variability, which has led to the definition of several genotypes. Thus for genogroups I and II, the most significant in humans, at least 8 and 17 genotypes respectively can be differentiated.

Sapoviruses (SaV) are non-enveloped viruses whose genome consists of a single-stranded RNA of positive polarity. They belong to the *Caliciviridae* family, genus *Sapovirus.* The genus *Sapovirus* includes five genogroups divided into several genotypes, among which four genogroups, I, II, IV and V, are pathogenic to humans.

#### 4.1.2. Disease

Caliciviruses (noroviruses and sapoviruses) are responsible for acute gastroenteritis (AGE). Noroviruses are the major agents of gastroenteritis in all age groups combined. Sapoviruses are responsible for AGE especially in young children (<3 years, particularly in crèches) and the elderly (particularly in retirement homes).

The gastroenteritis caused by these two viruses is characterised by a sudden onset of vomiting or diarrhoea after a short incubation period of 24 to 48 hours. The majority of infected people recover spontaneously within 2 to 3 days. The disease can however be complicated by dehydration with prostration, which significantly affects general health and may require hospitalisation or cause death, especially in the elderly or in those with chronic diseases (e.g. diabetes, cancer, patients on immunosuppressive therapy). A recent review of the literature (Patel *et al.*, 2008) showed that norovirus was found, according to the studies, in 5 to 36% of patients seen for AGE and in 5 to 31% of hospital inpatients. This virus thus seems to be the leading cause of AGE in adults and the second in children. Viral shedding in faeces is greatest between the first and third day after the onset of symptoms, and usually ends 3 to 7 days after the onset of illness. The amount of viral particles released into the environment is very high (around $10^6$ viral particles per ml of stools or vomited material).

Noroviruses can be detected in stools at low titres up to 8 weeks after the onset of symptoms in people without underlying disease (Atmar *et al.*, 2008). The study by Atmar with the prototype strain GI.I A 2T2 showed that the doses ingested (diluted stool) and causing the disease ranged from 4.8 to 48 RT-PCR units (Atmar *et al.*, 2008). Teunis *et al.*, also using human volunteers (and the same strain), developed a model and demonstrated the complexity of the infection in which different factors are involved, such as aggregation of viruses (which makes assessment of the infectious dose difficult) and blood group of individuals (some are not susceptible to infection) (Teunis *et al.*, 2008). There are a wide variety of responses to the same dose depending on the sensitivity of individuals (some not falling sick with a dose of 4800 RT-PCR units). Studies in healthy volunteers have shown that about 30% of infected people remain asymptomatic.

Noroviruses are transmitted in several ways:

- from person to person, through direct contact with an infected patient or indirect contact with a contaminated environment (faeces or vomit);
- dietary route:

- ingestion of food (consumed raw or without reheating) contaminated during preparation by a person shedding the virus;
- ingestion of water or food (shellfish, fruits or vegetables consumed raw, etc.) contaminated by discharge (sewage water, irrigation water, etc.) containing noroviruses;

- from droplets projected forcefully in the air by vomiting.

#### 4.1.3. Epidemiology of viral gastroenteritis (norovirus and sapovirus) related to the consumption of shellfish

Owing to the development of molecular biology methods since the 1990s, which have facilitated their identification, these viruses are now widely acknowledged as the principal agent in outbreaks transmitted by consumption of shellfish. Most outbreaks caused by these viruses occur in winter, at the same time as winter viral gastroenteritis outbreaks. They are due to consumption of raw shellfish (mostly oysters) collected from seawater contaminated with sewage or effluent (waste) from recreational or fishing boats (including the oyster boats). In these epidemics, the attack rate is high.

There are few published values on the norovirus concentration of oysters implicated in TIAC. In fact, most of the time it is very difficult to be certain that the oysters analysed actually correspond to those consumed. When these data exist, the minimum values found are variable: 25 to 85 RNA copies/g of oyster digestive tissue (Le Guyader *et al.*, 2010; Le Guyader *et al.*, 2003), with maximum values reaching 2500 RNA copies/g (Le Guyader *et al.*, 2008).

### 4.2. Contamination of the environment and of shellfish

#### 4.2.1. Origin of contamination

The presence of the virus in shellfish is usually due to contamination of the environment through wastewater discharges. When water purification plants are operating normally, discharges do not generally lead to high contamination of the receiving environment. Whenever there is heavy rainfall, however, hydraulic overloads in the plants may lead to discharges of wastewater that is less effectively treated, or even spills of raw sewage in the storm drains. The viral loads released into the environment are then far higher. Facilities that are not connected to the main sewage system, and camping cars and boats pumping out waste are other possible sources of contamination. Environmental compartments such as soils, sediments, groundwater and surface water, in which viruses may be retained and persist, are also potential secondary sources of viral contamination. However, the causes may also be more sporadic, diffuse, at a low rate or intermittent, which then makes it difficult to identify the origin.

Noroviruses and sapoviruses conveyed by sewage are highly resistant and persist after release into the environment, causing water contamination. They can aggregate on organic particles allowing them to persist in urban wastewater and resist sewage treatment (chlorine, ozone, UV), as well as in the environment and in shellfish.

#### 4.2.2. Persistence in shellfish

Shellfish, especially oysters which are filter feeders, concentrate the viral particles, which accumulate in their tissues. They can then cause infections and even epidemics among consumers of these products (when eaten raw or undercooked).

In the natural environment, the viruses persist for a very long time in shellfish. This was highlighted following an outbreak in France in 2002: noroviruses were detected by molecular biology in shellfish in the fishing area from which the shellfish responsible for the epidemic came, more than 2 months later (Le Guyader *et al.*, 2003). In 2006, in the Étang de Thau, following contamination caused by sewage, farmed oysters remained contaminated for one month (positive signal by molecular analysis) (Le Guyader *et al.*, 2008). During the AGE epidemic of winter 2009-2010, targeted

surveillance of certain French shellfish farming areas implicated in TIACs found evidence of calicivirus over several months. Thus in the Bay of Morlaix-Finistère, oysters remained contaminated from 1 March to 4 May 2010, and clams from the Petite Mer of Gâvres-Morbihan from 2 February to 26 April 2010 (unpublished IFREMER data). Furthermore, in Ireland, Doré *et al.* showed that the level of contamination of shellfish transferred to a clean area fell tenfold after 17 days, and a further four days in an aerated tank were needed for the norovirus concentration in these same shellfish to reach 100 genome copies/g (Doré *et al.*, 2010).

The depuration of shellfish in a conventional tank (unsinkable, aerated) also takes a very long time with respect to the elimination of the faecal indicator, *Escherichia coli.* Indeed, although it can be eliminated in one or two days, the virus can persist for several days or even a week or two (Pommepuy *et al.*, 2003; Richards *et al.*, 2010).

### 4.3. Surveillance and regulatory control

Compliance of shellfish growing products and processes currently relies on a bacterial contamination surveillance system. Quality criteria based on screening for *E. coli* are set by Directive 2006/113/EC[7]. This directive requires vulnerability profiles to be prepared for each shellfish production area.

This bacterial contamination surveillance system does not enable conclusions to be drawn as to the presence of viral contamination, due to the lack of correlation between the presence of the virus and that of the bacterial indicator.

To date, noroviruses and sapoviruses are not screened for in any surveillance system. However, random testing can be conducted in an alert situation, especially following the notification of human cases of gastroenteritis in which food is the suspected source.

Due to the lack of a validated, standard test method, no European regulatory microbiological criteria have so far been established for the virus, irrespective of the food matrix, but Regulation (EC) No. 2073/2005 considers it necessary to establish criteria applicable for viruses in live bivalve molluscs when the analytical methods are developed sufficiently (Preamble 27). A working group was established within the European Committee for Standardization in order to validate a horizontal method for detection of noroviruses and HAV in foods using real-time RT-PCR (the CEN/TC275/WG6/tag4 group). This current investigation is based on the results of various studies conducted by the reference laboratories and the European SeaFoodPlus programme (Pommepuy, 2007). In addition, a working group at EFSA (European Food Safety Authority[8]) is currently considering safe limits relating to noroviruses for the surveillance of oysters.

### 4.4. The case of the Étang de Thau

The site of the Étang de Thau covers about 7500 hectares and the water has a total volume of 260 million $m^3$. It is the largest and deepest lagoon on the French Mediterranean coast. Its average depth is 4.5 metres, and it reaches 10 metres in its central part (Serais, 2007). A 12 km-long sand barrier separates the lagoon from the Mediterranean, thus limiting exchanges between the lagoon and the sea.

Its 285 $km^2$ catchment area is drained by ten rivers, of which only one is permanent: the Vène. The flow of these temporary or permanent rivers may change very abruptly during more or less intense rainfall events such as the *épisodes Cévenols,* in autumn and spring. These floods lead to strong freshening of the water in the lagoon, which among other things is linked to microbiological contamination. An underground spring, the Vise, emerges north of the lagoon.

Exchanges with the sea are mainly through the canals of Sète in the northeast and secondarily by the Pisse-Saumes canal in the southwest. The lagoon is also linked to two other channels: the Canal du Rhône in Sète and the Canal du Midi.

[7] Directive 2006/113/EC of the European Parliament and of the Council of 12 December 2006 on the quality required of shellfish waters.
[8] EFSA Q-2010-00926: EFSA working group on Norovirus in oysters: methods, limits and control options.

The main vector for water circulation is the wind, due to the low volumes of incoming fresh water. Exchanges with the sea are governed by three factors: the astronomical tide, barometric tide and the wind (Serais, 2007).

The Étang de Thau accounts for 10% of overall French shellfish production and 90% of that in the Mediterranean (Serais, 2007). Approximately 12,000 tonnes of oysters and 3600 tonnes of mussels are produced on 840 suspension culture concessions. These concessions represent one fifth of the total area of the lagoon, along the northern coast and divided into three areas from East to West: Bouzigues, Mèze and Marseillan.

Urbanised land occupies 16% of the Étang de Thau's catchment area, which is above the national average for coastal areas. It has a population of 89,400, almost half of whom are concentrated on the town of Sète. The population density is about 240 inhabitants per km². The Étang de Thau has seen one of the largest recorded expansions on the French Mediterranean coast. Its tourism capacity is estimated at 1.2 to 1.3 million visitors per year.

### 4.4.1. Epidemiological situation

4.4.1.1 Summary of reported TIACs linked to consumption of oysters from the Étang de Thau

Shellfish from the Thau lagoon, and particularly oysters, have for many years been the source of a significant proportion of the TIACs linked to shellfish consumption reported in France. Thus, 28% (65/232) of these TIACs reported in France between 2000 and 2010 were explicitly attributed to consumption of oysters from the Étang de Thau, or occurred in the Languedoc-Roussillon region (Table 1). Even where the origin of the oysters on the mandatory notification (DO) [9]form is missing, they have been regarded here by approximation as TIACs potentially linked to consumption of oysters from the Étang de Thau (the main production site).

**Table 1: Reported TIACs attributed to consumption of oysters in the Languedoc-Roussillon region and in the rest of France from 2000 to 2010.**

| Year | Number ** | Languedoc-Roussillon* | Other regions | Total |
|---|---|---|---|---|
| **2000** | Outbreaks | 1 | 12 | **13** |
| | Cases | 4 | 143 | **147** |
| **2001** | Outbreaks | | 5 | **5** |
| | Cases | | 36 | **36** |
| **2002** | Outbreaks | 18 | 32 | **50** |
| | Cases | 67 | 45 | **112** |
| **2003** | Outbreaks | 4 | 5 | **9** |
| | Cases | 11 | 55 | **66** |
| **2004** | Outbreaks | | 4 | **4** |
| | Cases | | 45 | **45** |
| **2005** | Outbreaks | 1 | | **1** |
| | Cases | 6 | | **6** |
| **2006** | Outbreaks | 28 | 17 | **45** |
| | Cases | 201 | 258 | **459** |
| **2007** | Outbreaks | | 10 | **10** |
| | Cases | | 43 | **43** |
| **2008** | Outbreaks | 1 | 11 | **12** |
| | Cases | 4 | 176 | **180** |
| **2009** | Outbreaks | 7 | 11 | **18** |
| | Cases | 181 | 149 | **330** |
| **2010** | Outbreaks | 5 | 60 | **65** |
| | Cases | 41 | 414 | **455** |

[9] DO : déclaration obligatoire

| | | | | |
|---|---|---|---|---|
| **Total** | **Outbreaks** | **65** | **167** | **232** |
| | **Cases** | **515** | **1364** | **1879** |

Data from mandatory notifications (DO).
** clusters of TIACs linked to consumption of oysters, occurring in Languedoc-Roussillon (départements 11, 30, 34, 48, 66), or occurring in another region, but for which the investigation identified consumption of oysters originating from Thau-Bouzigues.*
*** Due to the significant under-reporting of TIACs in France, the actual number of TIACs linked to consumption of oysters, including those from the Étang de Thau, is probably much higher than the number of notified TIACs. This underestimation is also explained by the low numbers of patients seeking medical consultation at the onset of viral gastroenteritis (usually minor), who usually recover quickly and spontaneously.*

The most significant episodes of viral contamination involving many TIACs from enteric viruses (most commonly norovirus) took place in 2002/2003, 2005/2006, 2009 and 2010/2011. These episodes resulted in thorough epidemiological, microbiological and environmental investigations being conducted. They all occurred during a winter epidemic of viral gastroenteritis following periods of heavy rainfall, and were linked to contamination by several enteric viruses. They also highlighted the limitations of surveillance based on the faecal indicator *E. coli,* which failed to detect the viral contamination.

2002/2003 Episode (Barataud *et al*., 2003; Doyle *et al*., 2004)

Fourteen clusters of norovirus TIACs (90 cases) occurring between 14 December and 26 December 2002 were reported. Three different strains of norovirus (two from GGII and one from GGI) were identified in the stools of patients and two different strains (one from GGII and one from GGI) in oysters. This contamination episode occurred due to exceptional rainfall and flooding on 10 and 11 December 2002, which resulted in overflows from wastewater treatment plants (STEPs) and pumping stations in the catchment area. Analyses of oysters taken from three sites (Marseillan, Bouzigues and Mèze) between 16 and 19 December 2002 showed high contamination by *E. coli.* Following these results, a Prefectural Order instructed shellfish farmers to place the oysters in depuration tanks, a measure that was only partially implemented due to a shortage of facilities. No measures were taken to prohibit sale or withdraw shellfish. No TIACs linked to consumption of oysters from the Étang de Thau sold after December were declared in France. However, in January 2003, the Italian health authorities reported to the European rapid alert system for food and feed (RASFF) the occurrence of more than 200 cases of AGE in Italy, linked to the consumption of oysters from the Étang de Thau.

2005/2006 Episode (Barataud *et al*., 2003; Doyle *et al*., 2004; Faillie *et al*., 2007; Le Guyader *et al*., 2008; Le Guyader *et al*., 2009; Le Saux *et al*., 2009)

Thirty-eight clusters of norovirus TIACs (205 cases) occurred over a period of one month between 2 and 27 February 2006. Viral screening in the stools of patients and in oysters identified as multi-contamination by enteric viruses (noroviruses from several genogroups I and II, rotavirus, astrovirus, enterovirus and aichi virus).

Chronology of events:

- From 7 to 15 January 2006: peak of the winter 2005/2006 epidemic reached in Languedoc-Roussillon.
- From 23 to 29 January: very high rainfall (138.2 mm) in the Hérault *département*.
- 31 January: evidence of bacterial contamination in oyster samples taken from REMI sites (concentrations from 700 to 4800 *E. coli*/100g).
- 31 January: instruction by the Hérault prefecture to extend shellfish depuration times to a minimum of 15 days.
- 6 February: satisfactory results of REMI samples for *E. coli*.
- 7 February: first reports of TIACs occurring from 2 February.

- 10 February: instruction to withdraw all products sold between 30 January and 5 February.
- 20 February: withdrawal of all products sold between 28 January and 17 February following reports of new TIACs, subsequent to 5 February.
- After 17 February: reporting of new TIACs due to oysters, onset of TIACs reported until 27 February.
- 1 March: Prefectural Order prohibiting the sale of shellfish from the Étang de Thau (one month after confirmation of the REMI microbiological alert).
- 20 March: one positive analysis for norovirus at the detection limit from three samples tested.
- 23 March: ban lifted, the last reported TIAC due to oysters from the Étang de Thau having occurred on 27 February.

The various measures are shown in the figure below:

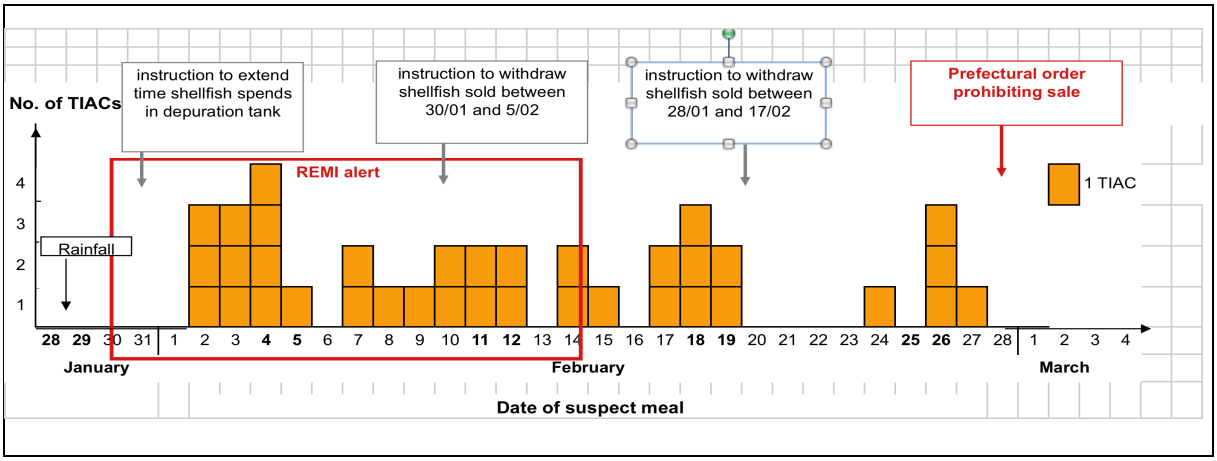



**Figure 1: Control measures implemented during the occurrence of TIACs associated with consumption of oysters from the Étang de Thau, France, January-February 2006**

During this episode, only the ban on sale, which was extended for 1 month until a negative result had been obtained from virus screening in shellfish and the AGE epidemic had declined, prevented the emergence of new TIACs.

2009 Episode

Six clusters of TIACs (68 cases) were reported following the consumption of oysters (four TIACs) or mussels (two TIACs) from the Étang de Thau at meals taken between 29 January and 25 February 2009. These TIACs occurred in six different *départements* (11, 13, 34, 56, 75 and 85). Virus screening carried out on the oysters and mussels responsible for the TIACs highlighted GGII noroviruses.

A level 0[10] REMI alert was triggered on 2 February, following heavy rainfall, and was maintained until 6 February 2009. Virus screening carried out at REMI stations on 19 February and 9 March 2009 yielded positive results for norovirus GGI and GGII at all the stations, combined with the presence of Aïchivirus at one station on 19 February. On 9 March, norovirus GGI alone was observed at one station, GGII alone at three stations and both GGI & GGII at two stations. No closure measure was implemented on the Étang de Thau.

[10] LEVEL 0: risk of contamination - alert triggered as a preventive measure
LEVEL 1: contamination detected - alert triggered on the basis of a result >= the alert threshold*
LEVEL 2: persistent contamination - alert triggered on the basis of 2 consecutive results >= the alert threshold*
*(*) Alert thresholds: Area A/1000 E. coli/100g of flesh and intervalvular fluid; Area B/4600 E. coli; Area C/46000 E. coli.*

Over this period, the only nearby production area closed[11] was the Étang du Prévost, on level 0 to 2 REMI alerts between 3 and 24 February and also implicated in three clusters of TIACs in the *département* of Le Puy de Dôme (63).

2010-2011 Episode

Eight clusters of TIACs responsible for 69 cases occurring between 24 and 26 December were reported following consumption of oysters from the Étang de Thau at meals taken between 24 and 25 December 2010 inclusive. These TIACs occurred in seven different *départements* (1, 11, 42, 69, 71, 74 and 77). All the cases reported clinical signs consistent with viral gastroenteritis. Virus screening performed in two patients from the same TIAC showed three different strains of norovirus: one GGIIb/GGII.13 strain, one 2010 variant GGII.4 strain and GGII.14. Virus screening carried out on the oysters at the origin of four of the TIACs revealed noroviruses and sapoviruses.

4.4.4.2. Situation of the AGE epidemic of winter 2010-2011 in Languedoc-Roussillon

According to data from INSERM's *Réseau Sentinelles* network, the regional epidemic of gastroenteritis began in Languedoc-Roussillon in mid-December 2010 (week 2010-50), with a regional epidemic peak being reached in the first week of January (week 2011-01) (see Figure 2). The epidemic observed this season has been severe. The estimated incidence at the epidemic peak (1268 cases per 100,000 inhabitants) was among the highest observed since 2004 (see Figure 2).

In week 2011-05, after seven weeks of epidemic, the incidence of gastroenteritis in Languedoc-Roussillon was estimated by the *Réseau Sentinelles* to be 226 cases per 100,000 inhabitants (CI 85 at 368 cases per 100,000 inhabitants). The number of cases estimated by this network decreased compared to the previous week and fell below the epidemic threshold set at national level. Note that this network announced the end of the gastroenteritis epidemic at national level for Week 5.

Surveillance conducted by the Languedoc-Roussillon Interregional Epidemiology Unit based on the activity of the emergency services (the InVS OSCOUR network) also showed a high number of visits to hospital emergency wards for gastroenteritis this year. Similarly, surveillance based on the activity of the voluntary associations *SOS Médecins* in Nîmes and Perpignan showed a high number of visits for this same reason. The data from these two networks confirm that the epidemic peak has passed, and show a decrease in the number of cases since the second week of January.

For week 2011-05, surveillance by *SOS Médecins* confirmed the decline in the number of gastroenteritis diagnoses recorded by its doctors, but with the number of weekly cases still higher than the 'non-epidemic' background. Regional data from the OSCOUR network nonetheless show that the number of visits to hospital emergency wards for gastroenteritis remained at a level that was still high and close to that of the previous week. This season has been marked by a large number of patients seeking emergency treatment for this reason.

[11] Order no. 2009-01-475 of 10 February 2009 temporarily prohibiting fishing, gathering and marketing of filter-feeding shellfish from the Étang du Prévost (shellfish growing area 34-26). (Repealed on 25/02/2009)

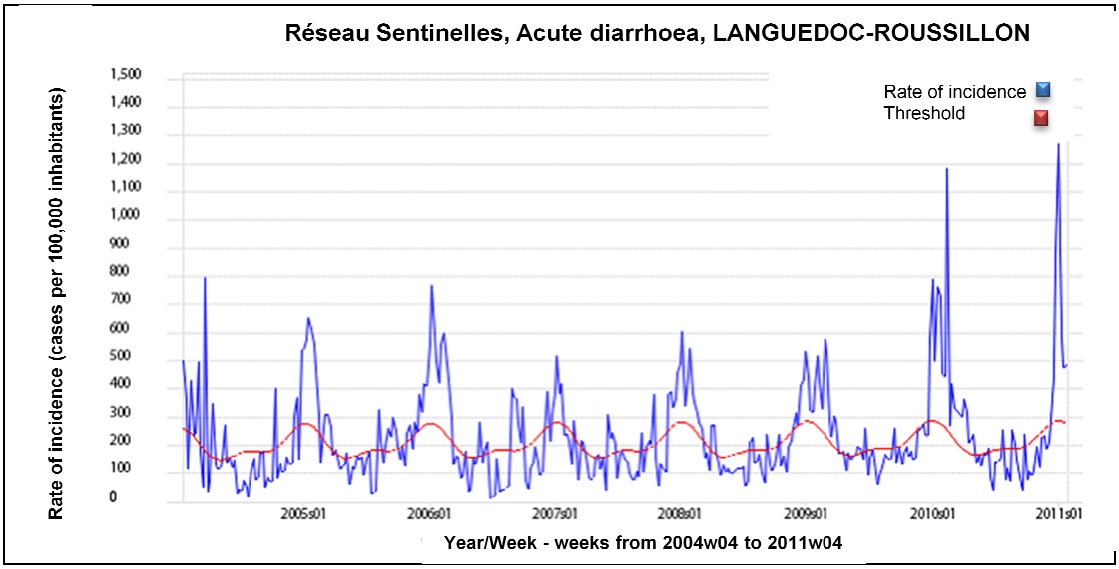



**Figure 2: Estimated incidence of acute diarrhoea in the Languedoc-Roussillon region. *Réseau Sentinelles* 2004-2011.**

In summary, the 2010-2011 gastroenteritis epidemic was severe in Languedoc-Roussillon. The epidemic peak was passed in the first week of January 2011. For week 5, the incidence estimated by INSERM's *Réseau Sentinelles* concurs with the end of the epidemic at national level. Estimates provided by the network at regional level confirm this. Surveillance based on the *SOS Médecins* networks and the emergency services still shows patients seeking treatment at a higher level than that observed in a non-epidemic period.

#### 4.4.2. Environmental conditions

The eight TIACs reported following consumption of oysters from the Étang de Thau concerned meals taken between 24 and 25 December 2010 inclusive.

The conditions preceding this period that would have led to viral contamination of the lagoon mainly relate to: the winter epidemic of acute gastroenteritis in the population, the rainfall and the malfunctions in the treatment system.

- Rainfall

Between 1 and 31 December 2010, periods of significant rainfall were observed (Météo France Sète station), with 39.9 mm/48h on 18 and 19 December, and 25.8 mm/72h from 21 to 23 December. During the period from 18 to 23 December, 65.7 mm of rain fell in 6 days.

This type of weather phenomenon is typical of the monthly distribution of rainfall episodes observed in this region (period from 1997 to 2006). Over these 10 years, for the month of January six episodes of rainfall between 10 and 30 mm were observed, and three episodes >40 mm.

- Malfunctions in the treatment facilities

Regarding sanitation, currently only one wastewater treatment plant, at Mèze-Loupian, discharges directly into the Étang de Thau, less than a kilometre from the concessions in the shellfish growing area. Since 18 October 2010, this lagooning station, which was already undersized, has been undergoing restructuring. The ongoing work caused a deterioration in the quality of water discharged, which was particularly evident during the rainfall episodes in December 2010 (data from the DREAL LR, 2 February 2011). This malfunction in the Mèze wastewater treatment plant was not reported in real time to the parties concerned.

However, when it rains, the lagoon is also polluted by spills from return pumping station overflows (12 catchment areas concerned), leaching from urban areas in seven catchment areas and discharges from private sewage treatment plants (1 catchment area concerned).

It should be noted that during earlier TIACs and especially in 2006, many pumping stations in the wastewater systems of coastal towns also malfunctioned during heavy rainfall, and discharged large amounts of untreated sewage directly into the lagoon: on 29 January 2006 the station at Balaruc released 14,000m$^3$ and the station at Marseillan released 132,000m$^3$. For the December 2010 event, there were malfunctions in the pumping stations, but none were reported in real time to the competent authorities or to shellfish growers.

The mean residence time for water in the lagoon is estimated at 94 days, making it a particularly confined environment compared to the Arcachon Basin (19 days). Residence time is heterogeneous (ranging from 44 days for the Petit Étang to 100 days in the southwest of the lagoon).

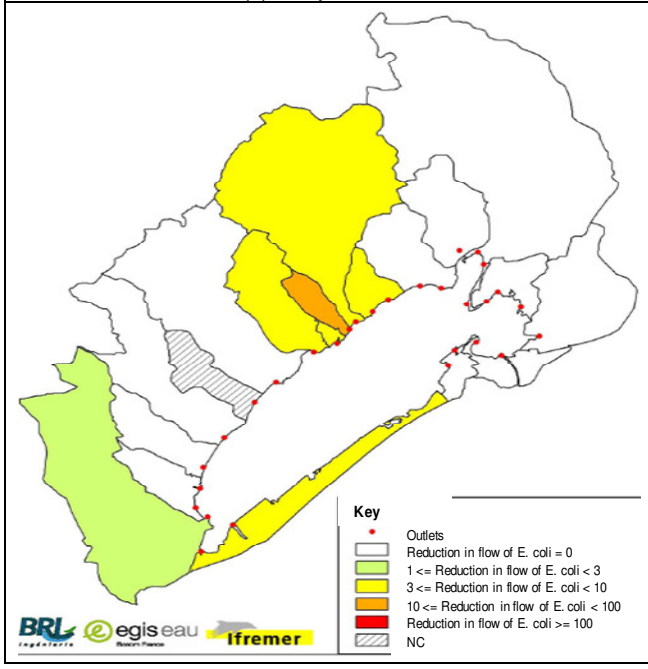
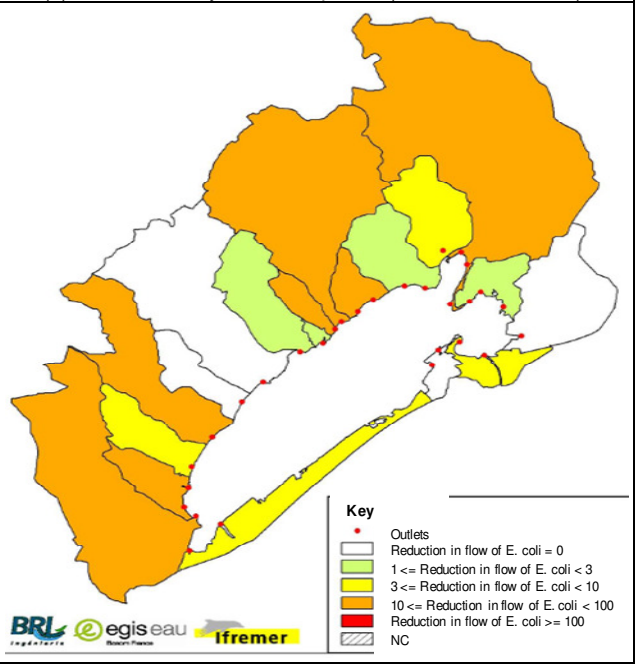
Given the fragility of the Étang de Thau it should be noted that under the Thau Quality Contract (*Contrat Qualité Thau*), whose objective is to restore the basin's microbiological quality, the OMEGA-Thau project (*Outil pour le Management Environnemental et la Gestion des Avertissements* - Tool for managing the environment and warnings) launched in 2007 and run by the Joint Association for the Étang de Thau (SMBT) aims to develop:

- a decision support tool for public investment in the catchment area (activity carried out from 2007 to 2010)
- and a system to anticipate risks of microbiological contamination for users (shellfish farmers, local authorities) and managers of the Étang de Thau (action deferred for now).

After making an inventory of sources of pollution and conducting a measurement campaign, validated modelling tools were used to establish a work programme for prioritising measures to be taken in the catchment area (BRLi**,** Egis Eau, IFREMER, 2010).

Maximum permitted flows (FMA) were determined for each outlet. The FMA is the maximum flow that the lagoon can tolerate, without harming the bacteriological quality of the water in the production areas, even under the most adverse weather conditions (Table 2).

**Table 2. Reduction in flows of *Escherichia coli* needed to maintain shellfish quality consistent with classification B in dry (a) and wet (b) weather (situation in 2008). Identification of contamination sources to be targeted.**

| (a) in dry weather | (b) rain for a two-year return period (total of 76 mm/24h) |
|---|---|
|  |  |

### 4.4.3.Shellfish contamination

From 20 December 2010, the REMI surveillance network declared an alert level 0 following the rainfall episode, which allowed the bacteriological quality of the nine REMI stations to be monitored. No result higher than 1200 *E. coli*/100g of flesh and intervalvular liquid was observed, which led to the alert being lifted on 21 December. On 3 January 2011 as part of routine surveillance, the REMI alert was again triggered by a score of 7200 *E. coli* at the Marseillan station. The subsequent results from 5 and 10 January showed an increasing contamination by *E. coli.* As of January 17 a decrease was observed, which was confirmed on the 24th, leading to the REMI alert being lifted on 25 January.

At the same time, qualitative norovirus and sapovirus contamination was observed at all nine stations. On 31 January (latest data available) four out of nine stations were negative for calicivirus. This pattern, corresponding to a slow viral decontamination of farmed shellfish, had already been observed in this lagoon during the 2006 episode (Le Guyader *et al.*, 2008).

Concerning the consumed shellfish implicated in the TIACs in the *départements* 42, 71, 74 and 77, and analysed by the National Reference Laboratory, the lack of information on traceability upstream of the shipping organisation means that it is impossible to identify the shellfish farming sector(s) affected: Bouzigues, Mèze or Marseillan.

| | | 20-Dec-10 | 3-Jan-11 | | 5-Jan-11 | 10-Jan-11 | | | 17-Jan-11 | | | 24-Jan-11 | | | 31-Jan-11 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | nov | sav | *E. coli* | *E. coli* | nov | sav | *E. coli* | nov | sav | *E. coli* | nov | sav | nov | sav |
| BOUZIGUES a | Mussels | 600 | GII | + | 850 | 1000 | GII | + | 1900 | GII | + | <130 | GI GII | + | GII | + |
| BOUZIGUES c | Pacific oysters | 580 | GII | + | <130 | 160 | GI GII | + | 160 | GI GII | + | <130 | GI | + | nég | + |
| PORT DE LOUPIAN | Pacific oysters | 190 | GII | + | <130 | <130 | GII | + | 1000 | nég | + | <130 | GII | + | nég | + |
| MEZE area a | Pacific oysters | 380 | GI GII | + | 550 | 1500 | GI GII | + | 340 | GII | + | <130 | GII | + | GII | nég |
| MOURRE-BLANC offshore | Pacific oysters | 270 | nég | + | 530 | 940 | GII | + | <130 | GI GII | + | <130 | GII | + | nég | nég |
| MEZE area b | Pacific oysters | <130 | GI | + | <130 | <130 | GII | + | <130 | GII | nég | 230 | nég | nég | nég | nég |
| MONTPENEDRE | Pacific oysters | <130 | GI GII | + | <130 | <130 | GI GII | + | <130 | nég | nég | <130 | nég | nég | nég | nég |
| LA FADEZE | Pacific oysters | 370 | GII | + | <130 | 170 | GII | + | 720 | nég | + | <130 | GII | + | nég | nég |
| MARSEILLAN offshore | Pacific oysters | 1200 | GII | + | 5500 | 20000 | nég | + | <130 | nég | nég | <130 | GI GII | + | GII | nég |

**Figure 3**: **Shellfish from the growing sector in the Étang de Thau and associated contamination**

| TIAC | Shellfish | | nov | sav | Rcvd Labo |
|---|---|---|---|---|---|
| 2011 42.01 | Oysters | consumed | + | - | 4-Jan-11 |
| 2011 77.01 | Oysters | consumed | + | - | 6 Jan 11 |
| 2010 74.13 | | | + | - | 4-Jan-11 |
| 2010 71.05 | Oysters | consumed | + | - | 4-Jan-11 |

★ No sample

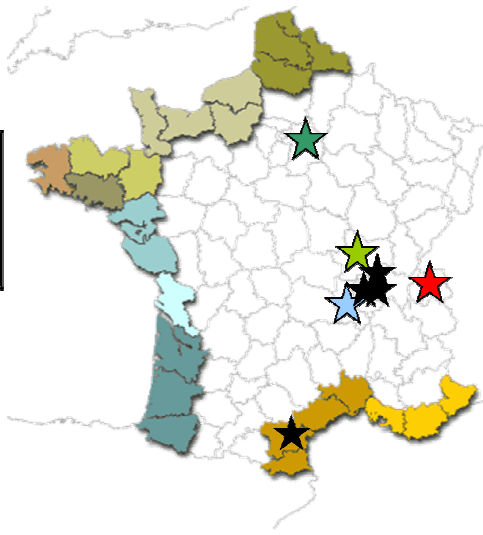


**Figure 4**: **Consumed shellfish implicated in the TIACs (from the Étang de Thau)**

The diagram below illustrates how the situation related to this episode developed, and the various factors involved (weekly incidence of AGE, *E. coli* results in treated water from the Mèze-Loupian lagooning station, rainfall, virological results from shellfish tested).

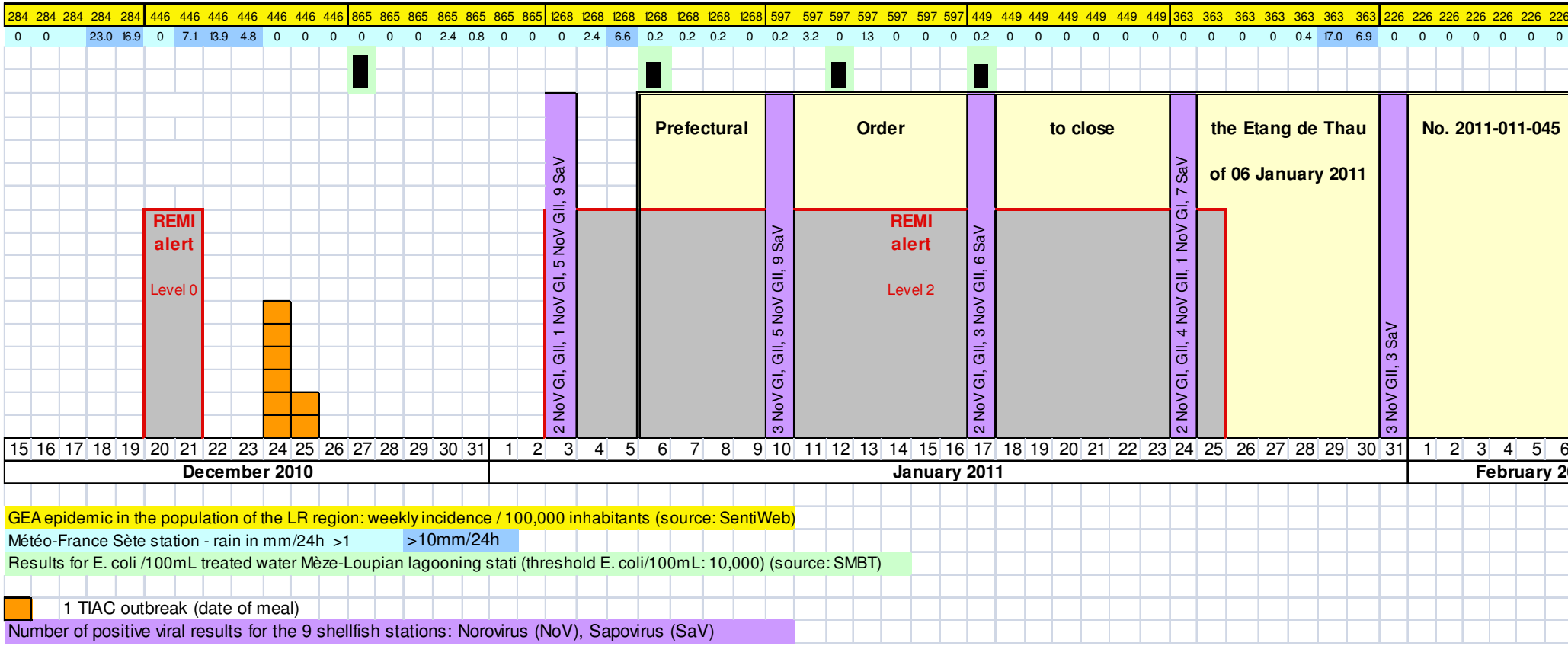



**Figure 5: Chronological information relating to the 2010/2011 TIAC episode**

### 4.5. Comparison of the situation in the Étang de Thau / Bay of Paimpol

In comparison, in the context of the surveillance measures for the Bay of Paimpol, reported outbreaks of hepatitis A (1999-2007) led the authorities to close the sector, and then reopen and monitor it. This surveillance identified the presence of norovirus, but in the absence of any TIACs the area was not closed. Over the past five years this shellfish farming sector has never been implicated in reported norovirus TIACs.

The Thau sector differs from that of Paimpol owing to a permanent population 5.5 times greater, with this population at Thau remaining high even in winter. During gastroenteritis epidemics, viral shedding pressure will be lower in Paimpol, and the receiving environment, which is open, will be more conducive to a reduction in viral load.

Moreover, there is probably a greater underestimation of TIACs in Thau than in Paimpol, due to greater awareness among health sector professionals following the hepatitis A epidemic.

### 4.6. Responses to questions raised:

- **What objective and relevant evidence can the competent authorities rely on to consider that shellfish from this area is safe for human consumption?**

✓ Interpretation of signals from RT-PCR screening as diagnostic information; quantification of viral particles

As the virus cannot be cultivated, only screening by molecular biology is possible. A few viral particles found in the shellfish (and measured by RT-PCR) are sufficient to induce gastroenteritis in a vulnerable person.

Studies in healthy volunteers have shown that the infectious dose for noroviruses is very low, and studies on the consumed oysters responsible for norovirus TIACs have shown that oysters with a low level of contamination (minimum values found from 25 to 85 RNA copies/g of oyster digestive tissue) can cause TIACs.

✓ Assessment of concomitant factors: weather, water temperature

The major rainfall episode extending from 18 to 23 December 2010 (65.7 mm in 6 days), which was accompanied among other things by the malfunctioning of the Mèze sewage treatment plant, is certainly the determining weather phenomenon that contributed to the contamination of the oysters responsible for the TIACs in late December 2010. The water temperature probably varied little over this period, but it was almost certainly low and therefore quite conducive to virus survival, given the observed minimum night-time air temperatures. The lagoon's low rate of water renewal should also be reiterated.

✓ Taking into account results obtained for norovirus at the different REMI points, when considering reopening

From the monitoring of the REMI points in the sector, a net decrease in the occurrence of viruses in shellfish was observed. Nevertheless, viruses (noroviruses and sapoviruses) are always present, and thus the risk remains greater than zero.

✓ Other relevant criteria to take into account

1. The development in the AGE epidemic situation in the population.

The regional gastroenteritis epidemic of winter 2010-2011 was particularly severe compared to previous years. The TIAC episode occurred at the beginning of this epidemic. Since this episode, the magnitude of the epidemic and the high population density in the Étang de Thau has resulted in large-scale, prolonged virus shedding in the environment. This may represent a significant reservoir of potential contamination for the lagoon's waters.

The epidemic peak was reached during the first week of January 2011. In week 2011-05, after seven weeks of epidemic, the incidence of gastroenteritis in Languedoc-Roussillon estimated by the

*Réseau Sentinelles* fell below the epidemic threshold set at national level. The other regional surveillance systems (*SOS Médecins* and the emergency services) showed that the number of patients seeking treatment was still higher than that observed in a non-epidemic period.

2. The operation of wastewater treatment plants and pumping stations.
3. The establishment of alert systems and the implementation of corresponding measures as stipulated by the recommendations of the OMEGA-Thau programme.
4. The control of the virus hazard by shellfish farmers, including traceability of their products.

- **Are the viral risk and the viruses' level of pathogenicity as high, several weeks after the closure of the area, as at the time of its closure further to the TIACs?**

In December 2010, the initial epidemic risk resulted from a combination of the start of the viral gastroenteritis epidemic in the population and heavy rainfall, resulting in highly contaminated wastewater being discharged by pumping stations and the wastewater treatment plant. The epidemic in the population is currently decreasing but there is still significant virus potential stored in the purification facilities. It is therefore essential to apply the existing alert criteria.

To our knowledge, there has been no episode of high rainfall nor malfunctioning treatment facilities since the epidemic episode in December 2010.

However, viruses are still present in the shellfish. The latest data available on 31 January 2011 (4 stations negative out of 9) show a slow viral decontamination of the farmed shellfish. No data are available on the virus's level of infectivity after four weeks in the marine environment.

In conclusion,

- The information available to date does not enable to conclude on a return to a safe situation, in particular due to the scale of the viral gastroenteritis epidemic which affected the region for 7 weeks and the persistence of positive calicivirus results in shellfish.
- However, the epidemic is now practically over, climatic conditions are more favourable, and the calicivirus results suggest a slow decontamination of the shellfish.
- It should be noted that in terms of measures, the 2006 episode showed that only the ban on sale, which was extended for 1 month until a negative result had been obtained from virus screening in shellfish and the AGE epidemic had declined, prevented the emergence of new TIACs.
- Shellfish depuration is a long and difficult process, so controlling the viral hazard requires careful assessment and a reduction in sources of contamination of the aquatic environment, in addition to the introduction of appropriate control measures for shellfish farming. As noted in a recent WHO report, protection of public health requires active monitoring of the water environment and the end product to ensure that controls are adequate (WHO, 2010). It is worth reiterating the importance of following the alert criteria and the application made by the OMEGA-Thau Project for alert systems to be set up.
- The importance of compliance with the regulatory requirements of traceability for these products should also be stressed. Furthermore, release analyses could provide information for assessing the safety of products intended to be placed on the market.

## 5. CONCLUSION

These are the points of analysis that the Agency is able to provide in response to the request from the DGAL for an opinion on a risk assessment relating to the reopening of a shellfish growing area closed due to the known presence of calicivirus (norovirus and sapovirus) in live shellfish.

**The Director General**

**Marc MORTUREUX**


## KEY WORDS

**Key words:** shellfish, virus, norovirus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