

POSSIBLE RELATION BETWEEN A WINTER EPIDEMIC OF ACUTE GASTROENTERITIS IN FRANCE AND VIRAL CONTAMINATION OF SHELLFISH

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ABSTRACT Several outbreaks of gastroenteritis related to the consumption of shellfish (frequently eaten raw) have been reported in different parts of the world. In Europe, human calicivirus infections may have been involved in winter outbreaks in recent years, although there is little evidence confirming such viral contamination in shellfish. This study presents the first results of a field survey on viral contamination in two shellfish harvesting areas along the French Mediterranean coast. The first, consisting mainly of oyster beds, was classified in category A, as determined by fecal coliform counts in shellfish (European Community Directive 91/492), and the second, a mussel bed, was classified in category C. Shellfish samples were collected monthly between August, 1995 and April, 1997, and RT-PCR was used to detect viruses known to be involved in outbreaks of gastroenteritis: enterovirus, human calicivirus, rotavirus, and astrovirus. Contamination by fecal coliforms was evaluated in the same samples. Virological results in shellfish were correlated with data on the incidence of epidemics of gastroenteritis in the coastal population obtained from a French survey. A relationship was observed between virological results and epidemiological data. For the 2 years when the incidence rate of gastroenteritis was maximal in winter, the mussel bed was always contaminated by the four types of viruses screened. Similar results were observed for oyster beds during the second winter; whereas, two samples were highly contaminated during the first winter, and a third showed low contamination (only rotavirus). These results suggest that an epidemic of gastroenteritis in the human population contributed to viral contamination of the marine environment through discharge of waste water.

KEY WORDS: shellfish, viral contamination, enterovirus, human calicivirus, rotavirus, astrovirus, fecal coliforms, gastroenteritis

INTRODUCTION

Enteric viruses are dangerous pathogens able to persist in the environment. They are introduced into marine and estuarine waters because current sewage-treatment practices are inadequate to eliminate them from wastewater effluents. Once in the environment, they can be accumulated by shellfish and lead to outbreaks among human consumers. Shellfish-borne diseases include hepatitis A, the most serious infection, which can prove fatal, and gastroenteritis, probably the most frequent pathology. Consequently, viral contamination of shellfish and shellfish farming areas has become a matter of increasing public health concern (Gerba and Goyal 1978, Metcalf et al. 1995).

In the 1980s, cell culture methods were used to detect enteroviruses (EV) in the tissues of clams and oysters in the United States (Vaughn et al. 1980, Wait et al. 1983). However, until recently, viral pathogens have rarely been identified in shellfish-associated outbreaks because of the lack of a sensitive method for detecting viruses not amplified in cell cultures. New analytical methods based on molecular biology now allow the detection of such enteric viruses as hepatitis A virus (HAV), human calicivirus (Hu CV), and rotavirus (RV) in environmental samples and shellfish implicated in food-borne outbreaks (Le Guyader et al. 1994, 1996a, Lees et al. 1995, Chung et al. 1996, Häfliger et al. 1997).

Some viruses detected in the coastal environment have been associated with gastroenteritis. Diarrhea is commonly reported among associated symptoms in infections by a number of enteroviruses (Melnick 1996). Hu CV, including Norwalk virus, have been responsible for gastroenteritis in children and adults, astroviruses (AV) are common causes of diarrhea in children, and RVs are the most important etiologic agents of severe diarrheal illness in in-

fant and young children (Kapikian 1996). This study investigated the occurrence of these viruses in shellfish collected over a 21-month period from Mediterranean beds. Virological results were correlated with data for the incidence of epidemics of gastroenteritis among the coastal French population and are considered in terms of their implications for public health.

MATERIALS AND METHODS

Environmental Sampling

Shellfish samples were collected monthly between August 1995 and April 1997 in two harvesting areas along the French Mediterranean coast. Site 1 was an oyster (*Crassostrea gigas*—Thunberg, 1793) bed producing 10,000 tons per year. Oysters were collected from three sampling points of this shellfish area classified as category A on the basis of fecal coliform counts (European Community Directive 91/492). Site 2, about 30 km from site 1, had a low mussel (*Mytilus galloprovincialis*—Lamarck, 1819) production of 50 tons per year. Mussels from this category C shellfish area were collected at a single sampling point.

Microbiological Analysis

Quantitative estimations of fecal indicators (*Escherichia coli*) in shellfish were performed by conductance measurement (Dupont et al. 1996). For viral analysis, shellfish were shucked and dissected. Viruses were eluted from digestive tissues and concentrated by precipitation. After proteinase K treatment, nucleic acids were purified and RT-PCR performed (Atmar et al. 1995). Amplification products were detected by electrophoresis and confirmed with specific probes. For EV, the region amplified by RT-

PCR corresponded to the 5' untranslated region; whereas, for Hu CV, it was the region coding for polymerase, for AV the 3' untranslated region, and for RV a portion of protein VP7 (Le Guyader et al. 1994, 1996b, Mitchell et al. 1995).

Data Analysis

Shellfish viral contamination was evaluated monthly at each sampling point according to an index of the frequency of gastroenteritis viruses calculated as follows:

$$I = [(V_{ev} + V_{cv} + V_{rv} + V_{av})/n] \times 100$$

where V for each virus (EV, Hu CV, RV, or AV) is expressed as positive (value of 1) or negative (value of 0); and n is the number of viruses analyzed in shellfish (n = 4).

Acute gastroenteritis in the French population was estimated using the French Sentinel System for Monitoring of Communicable Diseases (Flahault et al. 1995). Data on morbidity for seven communicable diseases were collected on a continuous basis via Videotex terminals from about 500 general practitioners. Results are expressed as the incidence rate per 100,000 inhabitants for acute diarrhea. These data were available on a local scale for this study.

RESULTS

The seasonal results for each virus detected among 83 samples analyzed are shown in Figure 1. Site 2 (category C) was contaminated much more heavily and frequently than site 1 (category A). However, the latter was not virus-free, and contamination was greater in autumn and winter, particularly in 1996. In site 1, RV were present in all samples collected during the winter of 1995, and Hu CV were detected simultaneously at all sampling points in the autumn of 1996. In site 2, Hu CV were only present in autumn and winter during both years.

Figure 2 shows the monthly occurrence of viral contamination from August 1995 to April 1997 based on the index of viral contamination expressed as the arithmetic mean of all sampling points (site 1 + site 2). Seasonal variations in pollution are apparent, with increases in autumn and a maximum in December and/or January, depending upon the year. Changes in bacterial contamination, expressed as the geometric mean of *E. coli* for all sampling points, show no apparent relationship between virus occurrence and coliform bacteria counts in shellfish. Similarly, bacterial contami-

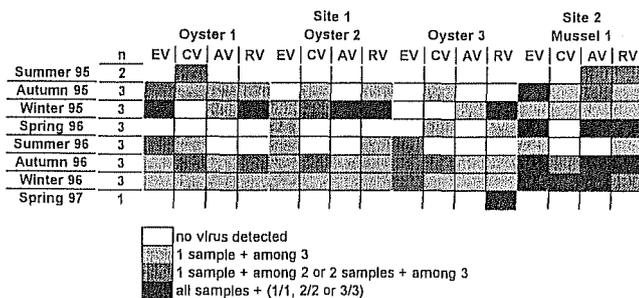


Figure 1. Viral contamination of shellfish between August 1995 and April 1997; Summer = July, August, September; Autumn = October, November, December; Winter = January, February, March; Spring = April, May, June; n = number of sampling months; EV, enterovirus; CV, human calicivirus; AV, astrovirus; RV, rotavirus.

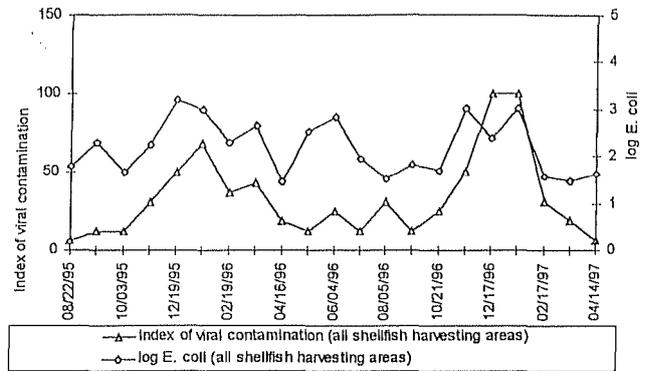


Figure 2. Comparison between index of viral contamination and shellfish bacterial contamination (all shellfish harvesting areas).

nation is apparently not correlated with the winter epidemic of acute gastroenteritis expressed as the local incidence rate per 100,000 inhabitants for acute diarrhea (Fig. 3). However, shellfish viral contamination seems to be correlated with the winter epidemic of acute gastroenteritis, with both indicators showing an increase in autumn and a peak in January (Fig. 4). This relationship was also observed for each site, as indicated for site 1 in Figure 5. Viral contamination was maximal in January, 1995, persisted until March, 1996, and was then apparent in December, 1996 and January, 1997. Results for site 2 did not show a clear relationship, probably because sampling was performed at a single point.

DISCUSSION

These results indicate that enteric viruses in shellfish were detected mainly in autumn and winter in the two sampling areas. No clear relationship was found between occurrences of viruses and coliform bacteria counts in shellfish. The variations in viral contamination seemed to follow the pattern of the winter epidemic of acute gastroenteritis in the French coastal population. These results suggest that the human epidemic contributed to viral contamination of the marine environment. However, our investigation, based on few data, provide only descriptive results and do not demonstrate a causal relationship. Further studies and more data are required to test the hypothesis statistically.

In France, large outbreaks of acute gastroenteritis occur every year during 4 or 6 weeks (end of December to January) (Flahault et al. 1997). This winter epidemic is caused mainly by a recent contact with someone with diarrhea but not by shellfish or tap

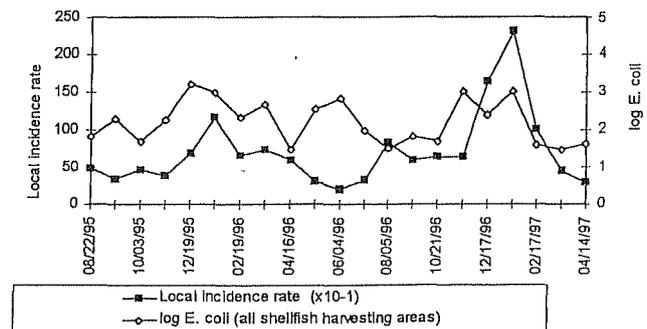


Figure 3. Comparison between winter epidemic of acute gastroenteritis and shellfish bacterial contamination (all shellfish harvesting areas).

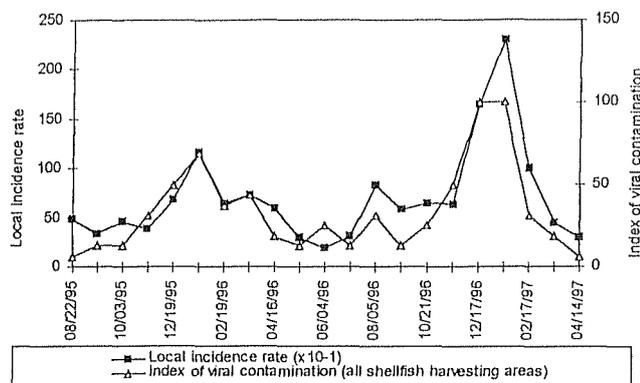


Figure 4. Comparison between winter epidemic of acute gastroenteritis and shellfish viral contamination (all shellfish harvesting areas).

water consumption (Létrilliart et al. 1997). Microbiological analyses have been unfrequently performed in stools, and few data are available on viral etiologic agents of diarrheal diseases (Maison et al. 1997). The proportion of each specific virus involved in these gastroenteritis epidemics remains unknown, but rotaviruses and caliciviruses are the most often reported etiologic agents. These viruses could be found in wastewater effluents (data not shown). Then, through sewage discharge, they could contaminate the marine environment (Dubois et al. 1997). The gradual changes of the enteric viruses in this environment are still unknown, and this question raises the problem of health hazards associated with shellfish consumption.

However, few data have been published in France on outbreaks of gastroenteritis related to shellfish consumption in winter. They occurred sporadically and locally. For example, one epidemiological study has demonstrated that an outbreak in December 1992 was statistically related to shellfish consumption. This epidemic, caused by a calicivirus, occurred in an area close to site 1, where the population was accustomed to eating local shellfish, which were the suspected vector for infection (Daurat 1992).

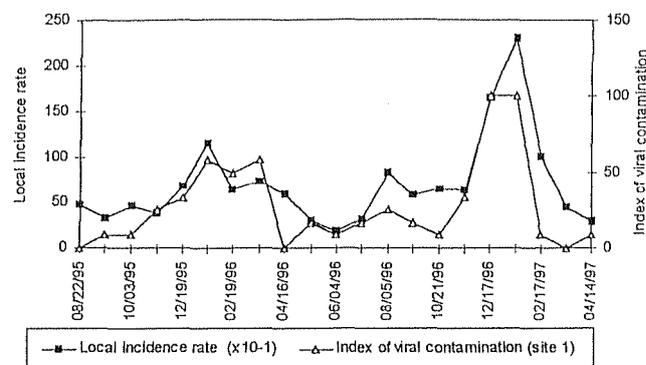


Figure 5. Comparison between winter epidemic of acute gastroenteritis and shellfish viral contamination (site 1).

Specific actions should prevent viral contamination of shellfish and reduce the public health hazards:

- (1) an efficient epidemic survey in the coastal population linked with a viral survey of sewage outputs in coastal environment;
- (2) the improvement of sewage treatment and, thus, of coastal water quality;
- (3) the respect of shellfish harvesting area classification in accordance with the European Community Directive. However, this regulation is based on the use of fecal coliform counts to assess the safety of shellfish beds, and this indicator is not an accurate reflection of shellfish viral contamination. Alternative indicators for viral pollution must be investigated and estimated according to enteric virus occurrence.
- (4) Shellfish depuration: more research is needed to improve depuration efficiency for removing viruses.

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