In: *Shellfish safety and quality*, (D. S. Shumways). University of Connecticut, USA, Dr Gary Rodrick, University of Florida, USA, pp. 108-128.

Chap 14: Monitoring viral contamination

M. Pommepuy, J. C. Le Saux, D. Hervio-Heath, S. F. Le Guyader

Introduction

Infectious diseases linked to the consumption of raw shellfish like oysters, mussels, cockles and clams, have long been identified. Bacterial diseases such as cholera and typhoid fever were the first to be suspected of being linked to consumption of contaminated shellfish (Butt *et al.*, 2004). During the past century, various strategies have been established in shellfish growing areas throughout the world to assure the sanitary quality of shellfish. More recently, despite sanitary surveys, *Vibrio parahaemolyticus, Vibrio vulnificus* and enteric viruses - especially Hepatitis A virus (HAV) and norovirus (NoV) – were found to be associated in outbreaks of human illness.

The pathogens involved in shellfish foodborne diseases can be placed in two classes. The first includes environmental pathogens that normally spend a substantial part of their life cycle outside human hosts, but which when introduced to humans cause disease with a measurable frequency (Cangelosi *et al.*, 2004). Among them, vibrios (*V. cholerae, V. parahaemolyticus, V. vulnificus*) are common in marine environmental infections, especially in countries where climatic conditions allow them to proliferate (Southeast US coast, South America and Asian countries), while few cases are reported in Europe. In the second class, the enteric pathogens are non-autochthonous microorganisms, discharged into the sea by raw or insufficiently treated waste waters during epidemics in the population. Most of the time they have been excreted by sick people living on coastal watersheds, but they may be present in the intestines of healthy humans or in the animal population (van der Poel *et al.*, 2001). Among them, viruses (especially NoV and HAV), are the chief concern in shellfish-borne diseases, while bacterial infections (salmonelloses, typhoid fever) have decreased thanks to sanitary control measures set up over the past century (development of detection methods and bacterial surveys and improvement of shellfish depuration technology).

This review will focus on the enteric viruses which are responsible for the main outbreaks linked to shellfish consumption (Butt *et al.,* 2004). Viral outbreaks associated with contaminated shellfish consumption was first suggested more than 50 years ago. Initially, the analysis of outbreaks was mainly based on epidemiological data and symptoms in patients (Mackowiack *et al*., 1976; Grohman *et al*., 1980; Richards, 1987). In some cases, microscopic studies identified small viruses in patients' stools or in shellfish (Appleton and Pereira, 1977; Morse *et al.,* 1986; Pontrefact *et al.,* 1993). The development of molecular biology and thus the ability to find low levels of enteric viruses in shellfish, has provided more accurate assessment of shellfish as disease transmission vehicles (Lees 2000; Sanchez *et al.*, 2002; Butt *et al.*, 2004; Boxman *et al.*, 2006; Le Guyader *et al.*, 1996, 2003, 2006). Despite the fact that many enteric viruses can be detected in human faeces (Metcalf *et al.,* 1995; A. Bosh et al, same book), only HAV and NoV have been clearly identified as infectious agents in consumed shellfish. Linking cases of viral disease to contaminated shellfish is not that easy, particularly for NoV, due to the multiplicity of lineages circulating at the same time (Blanton *et al.*, 2006; Zheng *et al.*, 2006).

One major drawback in shellfish outbreaks is the lack of consistent correlation between the indicator of fecal contamination (*E. coli* or fecal coliforms) and human enteric viruses and thus an absence of precaution (Lees, 2000; Butt *et al.,* 2004). In many viral outbreaks related to shellfish consumption, the level of *E. coli* was in compliance with the regulations (Christensen *et al.*, 1998; Le Guyader *et al.,* 2003, 2006; Kohn *et al.,* 1995; Boxman *et al.,* 2006).

Studies have been carried out in various countries to examine the prevalence of enteric viruses in shellfish. When we focus on data obtained from shellfish collected from producing areas or from the market, and showing no bacterial contamination as defined by current regulations, noroviruses were detected from 6-37% of the time (Hensilwood *et al.,* 1998; Le Guyader *et al.,* 2000; Beuret *et al.,* 2003; Nishida *et al.,* 2003; Cheng *et al.,* 2005; Costantini *et al.,* 2006). Different concentration and extraction methods and RT-PCR assays used, as well as the sampling season and the year of the study may explain the differences between the studies. It is also possible that prevalence surveys with positive findings may be over-represented due to a publication bias.

Some outbreaks have been linked to depurated oysters (Grohman *et al.,* 1980; Le Guyader *et al.,* 2006). Nonetheless, it must be kept in mind that while *E. coli* may disappear rather quickly either by depuration practices or natural cleansing, recent data show that viral depuration is difficult. Therefore, the two days of depuration stipulated by EC regulations is inefficient in eliminating viral contamination (Schwab *et al.,* 1998; De Medici *et al.*, 2001; Loisy *et al.,* 2005).

1. Identifying sources of pollution

In some outbreaks, multiple strains of a single virus such as norovirus can be detected indicating sewage or fecal contamination (Kageyama *et al.*, 2004; Gallimore *et al.*, 2005; Boxman *et al.,* 2006; Le Guyader *et al.,* 2006). Analysis of shellfish events leading to shellfish-related outbreaks has confirmed this hypothesis, and when environmental data are available, sewage-related contamination is often demonstrated (Table 1). Flooding has been shown to be responsible for viral contamination in other outbreaks and is congruent with the sudden introduction of multiple NoV strains into the oyster breeding site (Mackowiack *et al.,* 1976; Le Guyader *et al.*, 2006). In an attempt to identify the source of pollution in an oyster-producing area, Ueki *et al.*, (2005) did a one year-study collecting sewage, river water, oysters and clinical samples and looking for noroviruses. A clear impact of the sewage treatment plant was found on river contamination (about 75% of the sample being contaminated) and in shellfish samples (60% contaminated). Analysis of sequences obtained showed that the same diversity was observed among strains circulating in the population, as well as in sewage or in oyster samples, leading to the conclusion that improved sewage treatment is needed to guarantee the sanitary quality of these shellfish (Ueki *et al.*, 2005).

The literature review mainly highlights the human origin of viral shellfish outbreaks, i.e. the responsibility of urban wastewater. Nevertheless, in the state of current knowledge, animal sources cannot be ignored. For example, in the Netherlands, 44% of bovine fecal samples tested positive for norovirus (Van der Poel *et al.,* 2001). Even though inter-species exchange is extremely rare and has never been demonstrated for NoV, the high mutation rate could contribute to a species-crossing (Woolhouse, 2005). Differences between human and animal strains are very small (Dastjerdi *et al.,* 1999) thus, an intramolecular re-combination could lead to new pathogenic species (Lopman *et al.,* 2004).

2. Identifying the conditions responsible for microbial contamination of shellfish

In most of the polluted areas, evidence shows that the river and sewage outfalls discharging to the estuaries or marine bay have high levels of bacteria and viruses. Different sources of contamination are currently identified on sites where shellfish are farmed:

- Sewage discharges including sewage outfall, combined sewer overflows and stormwater discharges. The type of treatment applied to sewage waters plays an important role in the fecal load discharged into marine waters (physical, biological or tertiary treatments).
- Sewage network failures: many stormwater events could contribute to this pollution and could trigger persistent fecal contamination even during dry weather (Armstrong *et al.,* 1996).

- River discharges and possible run-off from agricultural activities (Kashefipour *et al.*, 2002; Crowther *et al.*, 2002; Vinter *et al.*, 2004)
- Other specific discharges could also come from boats, wild birds, bathers, sediments or other diffuse urban sources such as pigeons, dogs or cats (Sobsey *et al.*, 2003; Gerba 2000; Seifer *et al.*, 1997; O'Keefe *et al.*, 2005).

A high prevalence of human enteric viruses was reported in raw sewage with astrovirus concentrations ranging from $5x10^5$ to $5x10^7$ genome units (GU) /100ml (Le Cann *et al.*, 2004) or $3x10^3$ to $1.2x10^8$ genomes /l (Myrmel *et al.*, 2006). For norovirus, values varying from $3x10^1$ to $8.5x10^4$ GU /100ml (Lodder and Roda Husman, 2005), $1.8x10^4$ to $9.7x10^5$ genome/l (Myrmel *et al.*, 2006), less than 10^3 to 10^6 PCR detectable units/l (pdu/l) (van der Berg *et al.*, 2005) and up to $1.7x10^7$ genomes (G)/l (Laverick *et al.*, 2004) have been reported in different studies. In treated water, concentrations vary greatly depending on the location: $2x10^2$ to $5x10^4$ GU /100ml for astrovirus (Le Cann *et al.*, 2004), and for norovirus $4x10^1$ to $2.4x10^3$ GU /100ml (Lodder and Roda Husman, 2005), $1.6x10^5$ G/l (Laverick *et al.*, 2004), or $5.7x10^2$ pdu/l (van der Berg et al., 2005). Rivers can also be contaminated by norovirus and enterovirus (Schvoerer *et al.*, 2001; Hot *et al.*, 2003). On the basis of recent quantitative data, it was estimated that norovirus concentrations could reach $5x10^2$ GU/100ml in river (Lodder and Roda Husman, 2005), $1.6x10^3$ G/l (Laverick *et al.*, 2004). In floodwater sampled after a tropical storm event, Phanuwan *et al.*, (2006), found a high prevalence of viruses with a geometric mean of 14.0 pdu/ml for adenovirus, 13.0 pdu/ml (Hepatitis A virus), 5.3 pdu/ml (norovirus ggII), 0.7 pdu/ml (enterovirus) and 0.003 pdu/ml (norovirus ggI).

Seasonal variations in viral contamination were also reported. In the Meuse River, Westrell *et al.*, (2006) observed a distribution of norovirus with high peaks during winter (up to 1750 pdu/l) linked to contamination events in the catchments, especially sewage treatment failures. Noble and Furman (2001) observed the presence of enteroviruses in coastal water of Santa Monica Bay, California, especially during the winter wet season and the summer dry season. An interesting study by Haramoto *et al.,* (2005) showed a mean concentration of about 0.087 G/ml for genogroup I (ggI) norovirus and 0.61G/ml for ggII, with seasonal variations. In winter, these concentrations reached 0.21 G/ml for ggI and 2.3 G/ml for ggII. Lower concentrations were observed in summer (0.016 G/ml for ggI and 0.026 G/ml for ggII). For HAV, concentrations varying from 90 to 3523 copies/l were detected in estuarine waters along the Mexican borders (Brooks *et al.,* 2005). In some cases, seasonal patterns were observed reflecting the clinical epidemiology of the agent, in both river studies (Pusch *et al.,* 2005; van der Berg *et al.,* 2005) and a shellfish study (Le Guyader *et al.,* 2000).

The effect of rainfall on fecal water quality and on viral contamination has been also widely reported (Miossec *et al.,* 1998; Noble *et al.,* 2003; Haramoto *et al.,* 2006). Storm events cause land-based runoff and raw sewage overflow. Dramatic effects can be observed in developing countries with tropical climates and poor sewer systems. In Jakarta, Phanuwan *et al.* (2006) reported high concentrations of enterovirus, HAV and NoV in floodwaters after a storm event, leading to a high risk of viral infection for people who drink the water or who are in contact with overflow waters. In temperate or semi-arid climates, rainy storm events also lead to deterioration of seawater quality. Statistic observations showed that over half of the beach water quality failures in

Santa Monica Bay, California, were associated with rain events (Schiff *et al.*, 2003). Regional differences due to site-specificity also affect the impact of rainfall. In dry weather, Noble *et al.* (2001) observed that water quality standards were exceeded five times more often on Mexican beaches than on US beaches. In a multi-country study in Europe (Spain, Greece, Sweden, UK), using modelling approaches to compare NoV shellfish contamination, the country was found to be a significant input variable, and site-specific relationships between indicators and pathogens were highlighted (Brion *et al.*, 2004). Moreover, the increase in coastal populations contributes to physical changes in the landscape and leads to degradation of water quality. Mallin *et al.* (2001) demonstrated that the coastal demographic increase over a 14 year period in coastal North Carolina, was directly correlated with the upward trends in shellfish bed closures. In this region, population growth led to limiting of natural soil filtration by impermeable surfaces (e. g. roads, parking, roofs), new fecal sources and more rapid conveyance of pollutants to the sea. At the same time, in the same area, high sensitivity to forecast events was observed when compared with preserved coastal wetlands.

3. Potential strategies for reducing microbial contamination in shellfish harvesting areas

Over the last decade, coastal management has become increasingly important because of socio-economic requirements (tourism, aquaculture) leading to higher water quality objectives, and to increased pollution due to the upshift in coastal demography and industrial development. In addition to the complexity created by the topography in coastal catchments for raw wastewater collection, it is also difficult to have facilities which can cope with large seasonal variations in the fecal load. In some villages or small towns devoted to tourist activities, the population increases by 10-fold or more, for a few weeks in the summer season. Under these conditions, sewage treatment plants (STP) must deal with the need for increased capacity for just a few weeks in summer. In France, in coastal areas, primary treatment plants and activated sludge systems account for 17 and 61% respectively . The majority of small towns on the coast have been equipped only recently. Cities (>5,000 inhabitants) have set up tertiary treatment systems, which represent only 22% of the STPs located near the sea (IFEN, 2001).

3.1 How can fecal input be limited?

To limit pollution from urban wastewater, different types of treatments are now available. In general, processes are applied to eliminate the fecal load, but their real efficiency in eliminating viral contamination is often unknown. The very few data obtained under full scale conditions which have been reported in the literature are presented in Table 2. Primary treatment, decantation and coagulation have little effect on viral elimination, and secondary treatments -biological treatment with activated sludges, with/or without aeration or ponds- have a limited average efficiency (2 log and less). Tertiary treatments such as chlorination could make virus removal more efficient (Rose *et al.,* 1996). Effectiveness in removing viruses or phages can vary dramatically over time by a factor of 3 or 4 log, ranging from low values <1 log to 5-6 log viral reduction (Le Cann *et al.*, 2004; van der Berg *et al.*, 2005). Other factors could also be responsible for efficiency failures, for instance, the same processes may have different performances depending upon the age and capacity of the plant. Moreover, some families of viruses are more resistant than others to the processes used. Nevertheless, the high performances observed on some occasions in full scale STPs demonstrate that technology is capable of decreasing the viral load, and thus must be further investigated to improve the sea water quality.

Tertiary treatments should be investigated in depth, in order to promote modern solutions. Laboratory studies have provided information about the possible effect of disinfectants on viruses. Thus, a 2 log reduction of rotavirus and calicivirus was obtained with 19 mJ/cm^2 UV fluence (Hijnen *et al.*, 2006). Tree *et al.* (2003) demonstrated that 8 mg/l of free chlorine as sodium hypochlorite allows a 2.8 log poliovirus inactivation, but heavy doses of peracetic acid are necessary to reduce the poliovirus load (2 log reduction with 100ppm, 20 min) (Lazarova *et al.*,, 1999). Physical treatments such as membrane filtration, microfiltration or membrane bioreactors could also be promising: Ottoson *et al.* (2006) obtained a 3.78 log reduction for phages with a membrane reactor, Sano *et al.* (2006) demonstrated that ultrafiltration could physically eliminate NoV and polioviruses from sewage, and they estimated the $\log_{10}$ reduction values at more than 4 or 5.

Sewage management strategies to reduce the fecal input could lead to a real improvement of coastal water quality. Successful experiences are often observed but rarely reported in the literature. Figure 1 presents the evolution of *E. coli* concentrations in shellfish recorded on the same site in the Bay of Morlaix (France). The improvement was the result of efforts made by local decision-makers to improve the design of wastewater structures and facilities. Before 1992, both the sewer system and the treatment plant were obsolete: 30% of the raw water was directly discharged upstream of the estuary and failures in the sewer networks allowed raw water to be mixed directly in the river or in the estuary (2 km from shellfish beds). Despite tidal currents, the dispersion of contaminants was not sufficient for the shellfish growing area to comply with EU criteria, because of the raw fecal loads discharged upstream (Salomon and Pommepuy, 1990). After the wastewater network was rehabilitated, a decrease in the average concentration was observed. Whenever possible, rain water has been kept out of the sewer system, which has also led to a decrease in the pollution during rainfall events. A new wastewater treatment plant, for 58,000 equivalent/inhabitants with activated sludge, aeration and chlorination/de-chlorination treatments, was designed and built in February 1996. A recent study to assess the efficiency of the STP demonstrated that the *E. coli* removal efficiency of the STP is on average from 2.5 to 3 log for NoV (non published data). Since this date, the shellfish bed quality was recovered and *E. coli* concentrations fit the EU regulation level for the A classification.

An example of water quality recovery has also been reported in the Bilbao estuary (Garcia-Barcina *et al.,* 2006). In this area, over 10 years, a large part of raw wastewater discharges was intercepted and treated by the implementation of a biological treatment, leading to a fecal load reduction by 81.9%. As the result of the pollution abatement efforts to reduce the fecal load, improved seawater quality was observed and directly correlated to the capture of point-source discharges and the commissioning of a new STP. Another successful management concerns implementing remediation measures in a coastal area (Booth and Brion, 2004). By installing a force-main sewer line on Eagle Creek watersheds impacted by human activity, and collecting in over 60% of the houses in the villages, the input of untreated domestic sewage and storm overflow into the creek fell dramatically.

Observations after the sewer was installed clearly demonstrated that the remediation had been successful in removing significant sources of pathogens from the creek (Booth and Brion, 2004).

Even though it is difficult to predict long-term dynamics in the development of a region (Dominguez and Gujer, 2006), these examples demonstrated the capacity to remove fecal enteric contamination from urban sources and thus, to improve fecal water quality.

3. 2. Tools for management

For marine water quality recovery, reliable tools are needed to assess contamination sources on the coast and within the watersheds to define the priorities in terms of management. As stated above, all sources of contamination, including run-off from agriculture, have to be taken into consideration. Considerable progress has been made over the past decade for operationally developed models to predict coastal water quality. They are now running on watersheds to evaluate the impact of non-point fecal loadings and their role in local contamination, compared to urban contamination (Kay *et al.*, 2005a, 2005b). These models include land use and topographic data (Crowther *et al.*, 2003), forecast events (Jeng *et al.*, 2005), and livestock fecal load input (Crowther *et al.*, 2001, Viten *et al.*, 2004, Gannon *et al.*, 2005). There are also models for the transport and deposit of *E coli* associated with stream sediment (Jamieson *et al.*, 2005) as well as the behavior of free/attached bacteria (Garcia-Armisen *et al.*, 2006).

Modelling strategies to reduce microbial contamination in coastal areas take into account the fact that discharge of sewages to coastal waters induces variable contamination, which depends on the receiving waters's capacity to dilute the effluent (Rees *et al.*, 2000). Watershed models connected to hydrodynamic models (Faulkner *et al.*, 2000) can simulate the impact of stormwater runoff, diffuse point pollution or other sources on seawater quality (Wither *et al.*, 2005, Jeng *et al.*, 2005. Haramoto *et al.*2006). A recent application was devoted to assessing the impact of viral inputs on shellfish growing areas (Pommepuy *et al.*, 2002). Thus, models enable the impact of diverse pollutants, including pathogens to be simulated and are currently applied in coastal management.

Interestingly, the increasing use of models has led to research on predicting the risk of illness. The results considered a probable dose – response relationship that links fecal contamination concentrations in bathing areas to the likelihood of contracting gastro-enteritis (Harris *et al.*, 2004; Kay *et al.*, 2005a). The application of models is said to be a promising approach to test management strategies, for example, reducing specific sources impacting water quality, relocation of an outfall, or re-examining control strategies. They provide useful guidance on quantifying the health gain from various levels of treatment applied to waste waters (Harris *et al.*, 2004).

4. Improving risk management strategies for shellfish harvesting areas

Risk management strategy involves different measures to ensure consumer protection and a sustainable development of aquaculture. The former consists in enforcing the strict application of regulations. Research to assess the risk of shellfish consumption must be developed to improve safety control and consumer protection. Finally, warning systems could be set up in coastal areas to advise shellfish producers and administration officials when events could lead to shellfish contamination. These measures will ensure shellfish quality and increase consumer confidence and thus, would greatly contribute to sustainable development of aquaculture.

4.1 Applying the regulations

A set of marine sanitary regulations exists for shellfish and bathing water quality (EC Directive 91/492 for Harvesting Area Classification; European Bathing Directive 76/160/EEC). Moreover, regulations to protect coastal water in terms of its ecology, like the Clean Water Act (USA), or the European Framework Water Directive (FWD) must be applied to enhance water quality in terms of fecal pollution. Food Safety regulations complete the comprehensive legislative system to ensure consumer protection. If applied, all these regulations will lead to drastically improved coastal water quality where shellfish are grown.

The nested-regulation set up by lawmakers during the past decade refers to the competent authorities's responsibility to design tools and to establish measures to comply with standards, including those on catchments. The Drinking Water Directive published in 2004 by the World Health Organization also recommended a risk-based approach to the management of water quality from catchment to consumers. In the United States, in the Clean Water Act, and more recently in Europe in the FWD, it is up to the States to develop appropriate measures. Integrated river basin management has to be set up with coordinated programs of measures. Systems to protect all water bodies, surface water and ground water must be implemented (Blöch *et al.,* 2001; Vanrolleghem *et al.,* 2005). Although the FWD is addressing an ecological objective, this Directive has to take "protected areas" into account, among them shellfish growing areas or bathing areas governed by daughter Directives. Moreover, new regulatory catchment-based tools which have to be set up to assess pollution loads, could benefit in daughter Directive benefits. In this way, Budget models have already been developed and applied for bathing water quality assessment. (Kay *et al.,* 2005a).

Real improvements in shellfish water quality could be expected as the result of fecal load abatement efforts, such as rehabilitation of sewage networks and implementation of new STPs (Mallin *et al.,* 2001; Booth and Brion, 2004; Garcia-Barcina *et al.*, 2006). The strict application of the nested-regulations set up to preserve environment quality and safety could lead to a real upshift in water protection. Nevertheless, efforts are still needed to perfect the entire control system. Presently, although guideline values exist for marine water quality in Europe, there is no mandate for wastewater quality, unlike in the United States.

4. 2 Improved shellfish risk analysis

As a consequence of outbreaks of shellfish borne illnesses in the last century, most countries have developed regulations concerning the sanitary quality of shellfish growing areas (Anonymous, 2005; Zohrab, 2004; Anonymous, 2003). EC Directive 91/492 stipulates the classification of shellfish harvesting areas by *E. coli* standards in shellfish (< 230 *E. coli*). Shellfish waters in the United States are regulated by the Federal standard of 14 colony-forming units (CFU) per 100ml of water (USFDA, 1995). The same standards are applied in Australia, New Zealand and other areas where shellfish are grown.

Traditionally, quantification of risk relies on traditional fecal indicators *i.e.* fecal coliforms and *E. coli*. These standards have been shown to be suitable for the prevention of enteric outbreaks such as typhoid (Rose and Sobsey, 1993). However, as mentioned above, bacterial indicators are not suitable to predict all microbial risk. Quantitative information on pathogen concentrations in the environment will allow us to better evaluate health risks in coastal areas in developing quantitative risk assessment models. This approach is based on hazard identification, dose-response determination, exposure assessment and risk characterization. An application of this approach has been proposed by Rose and Sobsey (1993). Using an Echovirus 12 probability model, the individual risk for consumption of 60g of raw shellfish ranged from 2.2/10,000 to 35/1,000. For Bosch *et al* (1994), the rotavirus risk varied from 15/1000 to 540/1000 depending whether or not the shellfish was depurated.

Promising advances in molecular detection indicate the possibility of directly detecting the presence of targeted pathogens in the environment. Gene probes or biosensors are already available to detect pathogens in food (Scott *et al.* 2002,., Pommepuy & Le Guyader, 1998). Real Time PCR already provides quantitative information to implement a viral risk assessment approach for shellfish risk. These methods, applied to environment samples and combined with genetic information on strains (genotyping, molecular characterization...), could help identify the danger from pathogens amongst emerging pathogens.

4.3 Establishing a warning system

Different strategies have been proposed to protect the consumer, among them shellfish depuration (Lees, 2000). Unfortunately, enteric viruses are hard to remove from shellfish (Richards, 1988; Loisy *et al.*, 2005) possibly due to ionic binding with glycogen and mucus during the winter months (Burkhardt and Calci, 2000). Recently, Le Guyader *et al.* (2006) demonstrated Norwalk virus-specific binding to digestive tissues in the oyster by carbohydrate structures. This mechanism is similar to those used by this virus to attach to human histo-blood group antigens. These results explain why depurated shellfish which met the *E. coli* standards, were found to be involved in foodborne diseases (Butt *et al.*, 2004).

To overcome these difficulties, management strategies, as proposed above could be implemented to decrease the viral input and use marine dilution to grow shellfish in good water quality. Despite all the management measures which could be set up, specific events could still occur and lead to viral contamination. New management strategies could be to remove shellfish before the contamination when possible or if not, to rapidly warn shellfish producers about possible pollution events. An automated monitoring network has already been described to survey toxic algal blooms on a fish farm (Butler *et al.*, 2001) and the implementation of similar early warning systems was recently proposed for shellfish production sites (Le Saux *et al.*, 2006). Expert systems for monitoring wastewater treatment plants are already available and could be associated with prediction of wastewater inflow from sewage treatment plant (Punal *et al*, 2002). This information could be gathered through surveys for viral illness in the population (Pommepuy *et al.,* 2005). Once validated, early warning systems would be set up to send data from various probes deployed in the environment and provide real-time monitoring data on rainfall, STP failures, drops in salinity and other parameters (Le Saux *et al.,* 2006).

Recent changes in the frequency and intensity of extreme events have been reported by different authors (Pfister *et al.,* 2005; Chen *et al.,* 2006; He *et al.,* 2006). Forecast models devoted to climate change predict local modifications, which have to be taken into account in managing shellfish resources. If global warming brings about increased coastal rainfall, that could lead to increased runoff and fecal loading to coast waterways, the early warning system could be an important tool for developing both aquaculture and consumer confidence.

5. Conclusion and Future trends

Monitoring viral contamination is complex and must take into account different factors such as detection methods, technology, social demands and the sustainable development of aquaculture.

Recent advances in technology, especially in developing molecular tools, make it possible to look for pathogens directly in shellfish implicated in outbreaks. Searching for norovirus and Hepatitis A virus in seafood, for regulatory purposes, have been recommended by experts involved in EU, FAO and WHO working groups.

Quantitative data recently obtained on naturally contaminated samples implicated in outbreaks would help us better assess the threshold viral concentrations for regulations. In the near future, gene chip technology enabling direct detection of pathogens will contribute to managing the risk and preventing outbreaks (Rose & Grimes, 2001). Epidemiological networks are important to rapidly detect outbreaks and identify potential new pathogens.

Consumer demand nowadays is focused on food quality, and one of the monitoring objectives is to set up available regulations to avoid contaminated shellfish being put on the market. New regulations set up in Europe, the USA, or in other developed countries are taking this demand into account. Nevertheless, to complement the regulations, important information is lacking concerning risk assessment for shellfish consumption. For example, very little information exists on exposure to assess viral danger. Dose response, although probably very low, is still being studied.

The last aspect of monitoring concerns the sustainable development of aquaculture. This development is closely linked to environmental quality in shellfish breeding areas. The nested–regulations set up for water quality, bathing areas and shellfish growing areas, if well applied, would provide the guarantees and the management tools. The presence of shellfish growing areas can actually act as a guarantee of the protection of the environment. There are promising examples of coastal management designed to reduce the fecal load which could make recovery of water quality feasible. Used in association with early warning systems, they could help ensure shellfish quality and increase consumer confidence and thus, would greatly contribute to sustainable development of aquaculture. Shellfish have long been recognized as being beneficial to human health and this benefit should also be taken into consideration in managing the coastal areas and preserving the water quality.

Table 1: Analysis of shellfish events leading to outbreaks of human illness.

Table 2: Examples of reported performances of full scale sewage treatment plants on viral removal

Figure 1: Effect of setting up equipment on shellfish quality, Morlaix, France. Ref. REMI network, Ifremer data: www.ifremer.fr/envlit/ .

-♦- *Escherichia coli;* ■ Geometric mean (Gm)

References

Ang L H (1998), An outbreak of viral gastroenteritis associated with eating raw oysters, *Com Dis Pub Health*, 1, 38-40.

Anonymous (2005), A good practice guide to the (working document) from the EU Working Group on the microbiological monitoring of bivalve molluscan harvesting areas, European Union.

Anonymous (2003), Guide for the control of molluscan shellfish, Interstate Shellfish Sanotation Conference, National Shellfish Program US Department of Health and Human Service FDA, USA.

Appleton H and Pereira M S (1977), A possible virus aetiology in outbreaks of food-poisoning from cockles, *The Lancet*, 9, 780-781.

Armstrong I, Higham S, Hudson G, Colley T (1996), The beachwatch pollution monitoring programme: changing priorities to recognize changed circumstances *Marine Poll Bull*, 7-12, 249-259.

Beuret C, Baumgartner A, and Schluep J (2003), Virus-contaminated oysters: a three-month monitoring of oysters imported to Switzerland, *Appl Environ Microbiol*, 69, 2292-2297.

Blanton L H, Adams S M, Beard R S, Wei G, Bulens S N, Widdowson M A, Glass R I, and Monroe S (2006), Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000-2004 *J Infect Dis*, 193, 413-421.

Blöch, H. (2001). EU policy on nutrients emissions: legislation and implementation. *Wat Sci Techno.* 44, 1-6.

Booth J, Brion G M (2004), The utility of the AC/TC for watershed management: a case study, *Wat Sci Tech*, 50, 199-203.

Boxman I L A, Tilburg J J H C, te Loeke N A J M, Vennema H, Jonker K, de Boer E, and Koopmans M (2006), Detection of noroviruses in shellfish in the Netherlands, *Int J Food Microbiol,* 108, 391-396.

Brion G, Lingeriddy Neelakantan T R, Wang M, Girones R, Lees D, Allard A, Vantarakis A (2004), Probing Norwalk-like virus presence in shellfish, using artificial neural networks, *Wat Sci Tech,* 50, 125-129.

Brooks H A, Gersberg R M, Dhar A K (2005), Detection and quantification of hepatitis A virus in seawater via real-time RT-PCR, *J Virol Meth,* 127, 109-118.

Burkhardt W, Calci K R (2000), Selective accumulation may account for shellfish-associated viral illness, *Appl Environ Microbiol,* 66, 1375-1378.

Butler E C V, Blackburn S I, Clementson L A, Morgan P P, Parslow J S, and Volkman JK (2001), A survey strategy and environmental monitoring network for an estuary supporting finfish cage culture, *J Mar Sci,* 58, 460-468.

Butt A A, Aldridge K E, and Sanders C V (2004), Infections related to the ingestion of seafood Part I: viral and bacterial infections, *Lancet Inf Dis,* 4, 201-212.

Cangelosi G A, Freitag N E, Buckley M R (2004), From outside to inside: Environmental Microorganisms as human pathogens' American Academy of Microbiology colloquium, Feb 6-8, 2004, Washington, AAM.

Chen Z, Grasby S E, Osadetz K G, Fesko P. (2006). Historical climate ans stream flow trends and future water demand analysis in the Calgary region, Canada. *Wat Sci Tech*, 53, 1-11.

Cheng P K C, Wong D K K, Chung T W H, and Lim W W L (2005), Norovirus contamination found in oysters worldwide, *J Med Virol*, 76, 593-597.

Christensen B F, Lees D, Henshilwood K, Bjergskov T, and Green J (1998), Human enteric viruses in oysters causing a large outbreak of human food borne infection in 1996/97, *J Shellf Res*, 17, 1633-1635.

Costantini V, Loisy F, Joens L, Le Guyader F S, and Saif L J (2006), Human and animal enteric caliciviruses in oysters from different coastal regions of the United States, *Appl Env Microbiol*, 72, 1800-1809.

Christensen, B.F., Lees, D., Wood, K.H., Bjergskov, T., and Green, J. (1998), Human enteric viruses in oysters causing a large outbreak of human food borne infection in 1996/97, J. Shellfish Res. 17:1633-1635.

Crowther J, Kay D & Wyer M D (2002), Faecal-indicator concentrations in waters draining lowland pastoral catchments in the UK: Relationships with land use and farming practices, *Water Res*, 36, 1725-1734.

Crowther J, Wyer M D, Bradford M, Kay D, Francis C A. (2003). Modelling faecal indicator concentrations in large rural catchments using land ude and topographic data. *J Appl Microbiol*, 94, 962-973.

Dastjerdi A M, Green J, Gallimore C I, *et al* (1999) The bovine Newbury agent-2 is genetically more closely related to human SRSVs than to animal caliciviruses, *Virology*, 254, 1-5.

De Medici D, Ciccozzi M, Fiore A, Di Pasquale S, Parlato A, Ricci-Bitti P, Croci L (2001), Closed-circuit system for the depuration of mussels experimentally contaminated with hepatitis A virus, *J Food Prot* 64, 877-80.

Dominguez D, Gujer W. (2006). Evolution of a water treatment plant challenges traditional design concepts. *Water Res*, 40, 1389-1396.

El-Din G A, Smith D W (2002), A neuronal network model to predict the wastewater inflow incorporating rainfall events, *Water Res*, 36, 1115-1126.

E P A (1999), Review of potential modeling tools and approaches to support the BEACH program, Contract N° 68-C-98_010 US EPA 823-99-002, 68p and annexes

FaulknerH, Edmonds-Brown V, Green A. (2000). Problems of quality designation in diffusely polluted urban streams – the case of Pymme's Brook, north London. *Env Pollution*, 109, 91-107.

Gallimore C, Cheesbrough J S, Lamden K, Bingham C, Gray J (2005), Multiple norovirus genotypes characterised from an oyster-associated outbreak of gastroenteritis, *Int J Food Microbiol*, 103, 323-330.

Gannon VP, Duke GD, Thomas J E, VanLeewen J, Byrne J, Johnson D, Kienzle S W, Little J, Graham T, Selinger B. (2005). Use of in-stream reservoirs to reduce bacterial contamination of rural watersheds. *Sci Total Env*, 348, 19-31.

Garcia-Armisen T, Thouvenin B, Servais P (2006), Modelling the faecal coliforms dynamics in the Seine estuary France, *Wat Sci Tech*, 54, 177-184.

Garcia-Barcina J M, Gonzalez-Oreja J A, De la Sota A (2006,) Assessing the improvement of the Bilbao estuary water quality in response to pollution abatement measures, *Water Res*, 40, 951-960.

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

Goh K T, Chan L, Ding J L, and Oon C J (1984), An epidemic of cockles associated hepatitis A in Singapore, *Bull W H O*, 62, 893-897.

Grohmann G S, Greenberg H B, Welch B M, and Murphy A M (1980), Oyster-associated gastroenteritis in Australia: the detection of Norwalk virus and its antibody by immune electron microscopy and radioimmunoassay, *J Med Virol*, 6, 11-19.

Haramoto E, Katayama H, Oguma K, Ohgaki S (2005), Application of cation-coated filter method to detection of noroviruses, enteroviruses, adenoviruses, and torque teno viruses in the Tamagawa river in Japan, *Appl Env Microbiol,* 71, 2403-2411.

Haramoto E, Katayama H, Oguma K, Koibuchi Y, Furumai H, and Ohgaki S (2006), Effects of rainfall on the occurrence of human adenoviruses, total coliforms, and *Escherichia coli* in seawater, *Wat SciTech,* 54 (3), 225-230.

Harris E L, Falconner R A, Lin B (2004), Modelling hydroenvironmental and health risk assessment parameters along the South Wales Coast, *J Env Management*, 73, 61-70.

He J, Valeo C, Bouchart FJ-C. (2006). Enhancing urban infrastructure investistment planning practices for a changing climate, *Wat. Sci. Tech.*, 53, 13-20.

Henshilwood K, Green J, and Lees D N (1998), Monitoring the marine environment for small round structured viruses (SRSVs): a new approach to combating the transmission of these viruses by molluscan shellfish, *Wat Sci Tech,* 38, 51-56.

Hijnen W A W, Beerendonk E F, Medema G J (2006), Inactivation credit of UV radiation for virus, bacteria and protozoan (oo)cysts in water: a review, *Water Res* 40, 3-22.

Hot D, Legeay O, Jacques J, Gantzer C, Caudrelier Y, Guyard K, Lange M, Andréoletti L (2003), Detection of somatic phages, infectious enteroviruses and enterovirus genomes as indicators of human enteric viral pollution in surface water, *Water Res*, 37, 4703-4710.

IFEN (2001), Scees, enquête Eau, 2001.

Jeng H A, Englande A J, Bakeer R M, Bradford H B. (2005). Impact of urban stormwater runoff on estuarine environmental quality. *Est Coast Shelf Sci*, 63, 513-526.

Kageyama T, Shinohara M, Uchida K, Fukhusi S, Hoshino F B, Kojima S, Takai R, Oka T, Takeda N, and Katayama K (2004), Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to Norovirus in Japan, *J Clin Microbiol,* 42, 2988-2995.

Kashefipour S M, Lin B, Harris E, & Falconer R A (2002), Hydro-environmental modelling for bathing water compliance of an estuarine basin, *Water Res,* 36, 1854-1868.

Kay D, Wyer M, Crowther J, Stapleton C, Bradford M, McDonald A, Greaves J, Francis C, Watkins J. (2005a). Predicting faecal indicator fluxes using digital land use data in the UK’s sentinel water framework Directive catchment: the Ribble study. *Water Res.,* 39, 3967-3981.

Kay D, Wyer M, Crowther J, Wilkinson J, Stapleton C, Glass P. (2005b). Sustainable reduction in the flux of microbial compliance parameters from urban and arable land use to coastal bathing waters by a wetland ecosystem producedf by a marine flood defence structure, *Water Res.,* 39, 3320-3332.

Kay D, Fleisher J M, Salmon R L, Weyer M D, Godfree A F, Zelenauch-Jacquotte Z, Shore R (1994), Predicting likelihood of gastroenteritis from seabathing; results from a randomized exposure, *The Lancet,* 344, 905-909.

Kohn M A, Farley T A, Ando T, Curtis M, Willson S A, Monroe S S, Baron R C, Macfarland L M, and Glass R I (1995), An outbreak of Norwalk virus gastroenteritis associated with eating raw oysters, *JAMA*, 273, 466-471.

Laverick M A, Wyn-Jones A P, Carter M J (2004), Quantitative RT-PCR for the enumeration of noroviruses (Norwalk-like viruses) in water and sewage, *Let Appl Microbiol,* 39, 127-136.

Lazarova V, Savoye P, Janex M L, Blatchley E R, Pommepuy M (1999), Advanced wastewater disinfection technologies: state of the art and perspectives, *Wat Sci Tech,* 40 (4), 203-213.

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

Le Cann P, Ranarijaona S, Monpoheo S, Le Guyader F, Ferré V (2004), Quantification of human astroviruses in sewage using real-time RT-PCR, *Research in Microbiology,* 155, 11-15.

Le Guyader F, Haugarreau L, Miossec L, Dubois E, and Pommepuy M (2000), Three-year study to assess human enteric viruses in shellfish, *Appl Env Microbiol*, 66, 3241-3248.

Le Guyader F S, Neill F H, Dubois E, Bon F, Loisy F, Kohli E, Pommepuy M, and Atmar R L (2003), A semi-quantitative approach to estimate Norwalk-like virus contamination of oysters implicated in an outbreak, *Int J Food Microbiol,* 87, 107-112.

Le Guyader F S, Bon F, De Medici D, Parnaudeau S, Bertome A, *et al* (2006), Detection of noroviruses in an international gastroenteritis outbreak linked to oyster consumption, *J Clin Microbiol*,

Le Saux J.C., Derolez V., Brest G., Le Guyader F., Pommepuy M . 2006. Elaboration of a strategy to limit shellfish viral contamination*. in Molluscan Shellfish Safety*, Proceeding of the 5th International Conference on Molluscan Safety, Galway, edt Marine Institute, Ir. 342-349.

Lodder W J, de Roda Husman A M (2005), Presence of noroviruses and other enteric viruses in sewage and surface waters in the Netherlands, *Appl Env Microbiol*, 71, 1453-1461.

Loisy F, Atmar R L, Le Saux, J C, Cohen J, Caprais M P, Pommepuy M, and Le Guyader F S (2005), Rotavirus virus like particles as surrogates to evaluate virus persistence in shellfish, *Appl Env Microbiol*, 71, 6049-53.

Lopman B A, Vennema H, Kholi E, *et al* (2004), Increase in viral gastroenteritis outbreaks in Europe and epidemic spread of new norovirus variant, *The Lancet*, 363, 682-688.

Mackowiak P A, Caraway C T, Portnoy B L (1976), Oyster-associated hepatitis : lessons from the Louisiana experience, *Am J Epidemiol*, 103, 181-191.

Mallin M A, Ensign S H, McIver M R, Shank G C, Fowler P K (2001), Demographic, landscape, and meteorological factors controlling the microbial pollution of coastal waters, *Hydrobiologia*, 406, 185-193.

Metcalf T G, Melnick J L, and Estes M K (1995), Environmental Microbiology : from detection of virus in sewage and water by isolation to identification by molecular biology- a trip of over 50 years, *An Rev Microbiol*, 49, 461-487.

Miossec L, Le Guyader F, Haugarreau L, Comps M A, Pommepuy M (1998), Possible relation between a winter epidemic of acute gastroenteritis in France and viral contamination of shellfish, *J Shellfish Res*, 17 (5), 1661-1664.

Morse D L, Guzeich J J, Hanrahan J P (1986), Widespreead outbreaks of clam and oyster associated gastroenteritis Role of Norwalk virus, *N Eng J Med*, 3124, 678-68.

Murphy A M, Grohmann G S, Christopher P J, Lopez W A, Davey G R, and Millson R H (1979), An australian wide outbreak of gastroenteritis from oysters caused by Norwalk virus, *Med J Australia*, 2, 329-333.

Myrmel M, Berg E M M, Grinde B, Rimstad E (2006), Enteric viruses in inlet and outlet samples from sewage treatment plants, *J Wat Health*, 04, 197-209.

Ng T L, Chan P P, Phua T H, Loh J P, *et al* (2005), Oyster-associated outbreaks of norovirus gastroenteritis in Singapore, *J Infec,t* 51, 413-418.

Nishida T, Kimura H, Saitoh M, Shinohara M, Kato M, *et al* (2003), Detection quantitation, and phylogenic analysis of noroviruses in japanese oysters, *Appl Env Microbiol*, 69, 5782-5786.

Noble R T, Weisberg S B, Leecaster M K, McGee C D, Dorsey J H, Vainik P, and Orozco-Borbon V (2003), Storm effects on regional beach water quality along the southern California shoreline, *J Wat Heath*, 011, 23-31.

Noble R T, Fuhrman J A (2001), Enteroviruses detected by reverse transcriptase polymerase chain reaction from coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels, *Hydrobiologia*, 460, 175-184.

O'Keefe B, D'Arcy B J, Davidson J, Barbarito B, and Clelland B (2005), Urban diffuse sources of faecal indicators, *Wat SciTech*, 51 (3-4), 183-190.

O'Mahony M, Gooch C D, Smyth D A, Trussel A J, Bartlett C L R, Noah N D (1983), Epidemic hepatitis A from cockles, *The Lancet*, 5, 518-520.

Ottoson J, Hansen A, Bjorlenius B, Stenstrom T A (2006), Removal of viruses, parasisitc protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant, *Water Res*, 40, 1449-1457.

Phanuwan C, Takizawa S, Oguma K, Katayama H, Yunika A, Ohgaki S (2006), Monitoring of human enteric viruses and coliform bacteria in waters after urban flood in Jakarta, Indonesia, *Wat ScTech*, 54, 203-210.

Pfister L, Drogue G, Poirier C, Hoffmann L. (2005). Spatial variability of trends in hydrological extremes induced of orographycally enhanced rainfall events due to westerly atmospheric conditions, *Wat Sci Tech*, 51, 15-21.

Pommepuy M, Hervio-Heath D, Caprais M P, Gourmelon M, Le Saux J C, Le Guyader F (2005), ‘Fecal contamination in coastal area: an engineering approach’ *In* Oceans and Health: Pathogens in the marine environment, (S Belkin Eds) Kluwer Academic/Plenum Publishers, USA, 331-360

Pommepuy M, Dumas F, Caprais M P, Camus P, Le Mennec C, Parnaudeau S, Haugarreau L, Sarrette B, Vilagenes P, Pothier P, Kohli E, Le Guyader F (2004), Sewage impact on shellfish microbial contamination, *Wat Sci Tech,* 50, 117-124.

Pommepuy M, & Le Guyader F (1998), Molecular approaches to measuring microbial marine pollution, *Current Opinion in Biotechnology,* 9, 292-299.

Pontrefac R D, Bishai F R, Hockin, J, Bergeron G, and Parent R (1993), Norwalk like viruses associated with a gastroenteritis outbreak following oyster consumption, *J Food Prot,* 56, 604-607.

Punal A, Roca E, & Lema J M (2002), An expert system for monitoring and diagnosis of anaerobic wasterwater treatment plants, *Water Res,* 36, 2656-2666.

Pusch D, Oh D Y, Wolf S, Dumke R, Schroter-Bobsin U, Hohne M, Roske I, Schreier E (2005), Detection of enteric viruses and bacterial indicators in German environmental waters, *Arch Virol*, 150, 929-947.

Richards G P (1987), Shellfish associated enteric virus illness in the United states, 1934-1984, *Estuaries,* 10, 84-85.

Rees G, Bartram J, Pond K, Goyet S (2000), Chapter 1: Introduction *in* Monitoring Bathing waters: A practical guide to the design and implementation of Assessments and monitoring Programmes, WHO, 1-11.

Rose J B, Dickson L J, Farrha S R, Carnahan R P (1996), Removal of pathogenic and indicator microorganisms by full-scale water reclamation facility, *Wat Res,* 30, 2785-2797.

Rose M A, Dhar A K, Brooks H, Zecchini F, Gersberg R M (2006), Quantification of hepatitis A virus and enterovirus levels in the lagoon canals and Lido beach of Venice, Italy, using real time PCR, *Water Res*, 40, 2387-2396.

Rose J B, & Grimes D J (2001), Reevaluation of microbial water quality: powerful new tools for detection and risk assessment, *A report from the American Academy of Microbiology.*

Rose J B, & Sobsey M D (1993), Quantitative risk assessment for viral contamination of shellfish and coastal waters, *Journal of Food Protection*, 56 (12), 1043-1050.

Salomon J C, Pommepuy M (1990), Mathematical model of bacterial contamination of the Morlaix estuary (France), *Water Res*, 24, 983-994.

Sanchez G, Pinto R, Vanaclocha H, and Bosch A (2002), Molecular characterization of hepatitis A virus isolates from a transcontinental shellfish-borne outbreak, *J Clin Microbiol*, 40, 4148-4155.

Sano D, Ueki Y, Watanabe T, Omura T (2006), Membrane separation of indegenous noroviruses from sewage sludge and treated wastewater, *Wat Sci Tech*, 54, 77-82.

Schwab K J, Neill F H, Estes M K, *et al* (1998), Distribution of Norwalk virus within shellfish following bioaccumulation and subsequent depuration by detection using RT-PCR, *J Food Prot*, 61, 1674-80.

Schiff K C, Morton J, Weisberg S B. (2003). Retrospective evaluation of shoreline water quality along Santa Monica bays beaches. *Mar. Environ. Res.* 56, 225-243.

Schvoerer E, Ventura M, Dubois O, Cazaux G, Serceau R, Gournier N, Dubois V, Caminade P, Fleury H J A, Lafon M E (2001), Qualitative and quantitative molecular detection of enteroviruses in water from bathing areas from a sewage treatment plant, *Res Microbiol*, 152, 179-186.

Scott T M, Rose J B, Jenkins T M, Farrah S R, & Lukasik J (2002), Microbial source tracking: Current methodology and future directions, *Appl Env Microiol* 68, 5796-5803.

Seyfried P L, Choi B C K, & Zhou R H (1997), Factors affecting fecal concentrations in water and sediment at various geographical locations on Georgian bay, Ontario, Canada, *Ecosystem Health,* 3, 107-114.

Sobsey M D, Perdue R, Overton M, & Fisher J (2003), Factors influencing feacal contamination in coastal marinas, *Wat Sci Tech*, 47, 199-204.

Stafford R, Strain D, Heymer M, Smith C, Trent M, and Beard J (1997), An outbreak of norwalk virus gastroenteritis following consumption of oysters, *Comm. Diseases Intellig.*, 21, 317-322.

Tree J A, Adams M R, Lees D N (2003), Chlorination of indicator bacteria and viruses in primary sewage effluent, *Appl Env Microbiol,* 69, 2038-2043.

Turner S J, Lewis G D (1995), Comparison of F-specific bacteriophages, enterococci, and faecal coliforms densities through a wastewater treatment process employing oxidation ponds, *Wat SciTech*, 31, 85-89.

Ueki Y, Sano D, Watanabe T, Akiyama K, Omura T (2005), Norovirus pathway in water environment estimated by genetic analysis of strains from patients of gatsroenteritis, sewage, treated wastewater, river water and oysters, *Water Res,* 39, 4271-4280.

Van der Berg H, Lodder W, van der Poel W, Vennempa H, de Roda Husman A M (2005), Genetic diversity of noroviruses in raw and treated sewage water, *Res Microbiol,* 156, 532-540.

Van de Poel W, van der Heide R, Verschoor F, *et al* (2001), Epidemiology of Norwalk like virus infections in cattle in The Netherlands, *Vet Microbiol,* 297-309.

Vanrolleghem P A, Benedetti L, Meirlaen J (2005), Modelling and real-time control of integrated urban wastewater system, *Env Model Software*, 20, 427-442.

Vinten A J A, Lewis D R, McGechan M, Duncan A, Aitken M, Hill C, Crawford C (2004), Predicting the effect of livestock inputs of *E coli* on microbiological compliance of bathing waters, *Water Res*, 38, 3215-3224.

Westrell T, Teunis P, van der Berd H, Lodder W, Ketelaars H, Stenstrom T A, de Roda Husman A M (2006), Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period, *Water Res,* 40, 2613-2620.

Wither A, Greaves J, Dunhill I, Wyer M, Stapleton C, Kay D, Humphrey N, Watkins J, Francis C, McDonald A, Crowther J. (2005). Estimation of diffuse and point source microbial pollution in the Ribble catchment discharging to bathing waters in the north west of England.

Woolhouse M E, Growtage-Sequeria S (2005), Host range and emerging and reemerging pathogens, *Emerg Inf Dis,* 11, 1842-1847.

Xu, Z Y, Li Z H, Wang J X, Xiao Z P, and Dong D X (1992), Ecology and prevention of a shellfish associated hepatitis A epidemic an Shangai China, *Vaccine,* 10, 567-568.

Zheng D P, Ando T, Fankhauser R L, Beard R S, Glass R, and Monroe S S (2006), Norovirus classification and proposed strain nomenclature, *Virology,* 346, 312-323.

Zohrab T (2004), Animals Products (specifications for bivalve molluscan shellfish), Notice 2004 FDA, New Zealand.