Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept

EU Integrated Project no 506359

Control of public health risks associated with sewage-contaminated shellfish

A summary of current control measures and technical developments

Bill Doré

SEAFOODplus Publication Series
Report 3.0.0 - 2007
ISBN 978-87-7075-002-8

Health promoting, safe seafood of high eating quality in a consumer driven fork-to-farm concept

EU Integrated Project no 506359

A report from RTD area 3

'Seafood safety'

Control of public health risks associated with sewage-contaminated shellfish

A summary of current control measures and technical developments

2007

Bill Doré
Marine Institute
Rinville, Oranmore
Co. Galway
Ireland

SEAFOODplus Publication Series
Report 3.0.0 - 2007
ISBN 978-87-7075-002-8

SUMMARY

- Bivalve Molluscan Shellfish represent a significant public health risk when harvested from sewage-contaminated waters. Such shellfish may be contaminated by a wide range of human pathogenic organisms.

- Current European controls and approved treatment processes (apart from heat treatment) reduce but do not eliminate the risks associated with sewage contaminated bivalve molluscan shellfish. The major risks associated with treated shellfish are gastroenteritis caused by Norovirus (NoV) and infectious hepatitis caused by hepatitis A virus (HAV).

- Recent technical advances in virus detection methods in shellfish mean that the introduction of viral standards in bivalve shellfish and the prospect of routine viral monitoring in shellfish harvesting areas can be realistically considered for the first time. Efforts within Europe at standardisation of real-time PCR methods for virus detection are well advanced and include A European Committee for Standardization (CEN) working group on developing standardised virus methods for food, the SEAFOODplus research project REFHEPA and ring-trials conducted by the Community Reference Laboratory.

- Work on developing risk-based management procedures in European shellfish is being conducted. This allied to viral monitoring has the potential to significantly increase the safety associated with of bivalve shellfish being placed on the market. However the complexity of the factors affecting viral contamination in shellfisheries and the limitation of current intervention measures available to control the identified risk must be considered when developing this work further.

- Technological advances in virus detection methods and greater understanding of the risk factors involved in viral contamination of shellfish are being achieved. These have the potential to significantly increase the level of safety associated with bivalve Molluscan shellfish. However, the implementation and use of this knowledge requires careful consideration by risk managers at all levels and in the broadest sense including industry, regulators and scientists.

SCOPE

This document gives an account of the current state of knowledge with regard to the health risks associated with the consumption of sewage-contaminated shellfish In a European context. The document aims to provide factual information on the current knowledge to relevant stakeholders;

- The viral risks associated with bivalve molluscan shellfish consumption.
- Current legislative procedures and treatment processes aimed at controlling the risk.
- The effectiveness of the current controls and treatment processes.
- Recent advances in detection methods for viruses in shellfish and risk management procedures

INTRODUCTION

Bivalve molluscan shellfish such as oysters, mussels, cockles and clams feed by filtering large volumes of seawater. During this process bivalve shellfish can accumulate and concentrate microbiological pathogens when grown in sewage-polluted harvesting areas. Such shellfish represent a significant risk when consumed raw or lightly cooked.

There are range of microbiological pathogens found in sewage and consequently in sewage-contaminated shellfish. This includes bacteria, parasites and viruses. The significance of shellfish as a vehicle for transmitting illness was first recognised at the end of the 19th Century when a number of well documented outbreaks of Typhoid Fever were observed in the USA, UK and France. In response, over the next 100 years or so various control measures have been introduced in most developed countries to prevent such illness. These have developed into the legislative controls that we see in place today across the European Union (EU). These controls have been very effective at reducing the degree of bacterial illness associated with shellfish consumption to very low levels. Despite this success there remains a significant risk of viral illness associated with bivalve shellfish consumption even in shellfish compliant with all legislative and microbiological standards. Principal among these illness are gastro-enteritis caused by norovirus (NV) and infectious hepatitis caused by hepatitis A virus (HAV). Further details of the characteristics of these viruses and the illness they cause are given in Appendix 1.

It is therefore well recognised that further controls are needed to reduce the incidence of viral illness associated with bivalve shellfish. Until recently, efforts to control these viral risks have been hampered by a lack of methods to detect human pathogenic viruses in shellfish. This paper gives an account of the current controls in place in Europe, their effectiveness at preventing viral illness and the current state of knowledge moving forward to develop improved safety procedures.

CURRENT LEGISLATIVE CONTROLS

EU Regulation

Extensive EU regulations have been in place for almost 15 years to control the health risks associated with the consumption of BMS. EU directive 91/492 laid down the health conditions for the production and the placing on the market of live bivalve molluscs. EU directive 91/492 was just one of 17 vertical directives relating to food safety which were developed in the EU since 1964. Following the publication of a European white paper on food safety in 2000 a review these 17 directives was carried out by the European Commission. The review recognised that the high number of these directives and their individual complexity was unnecessary and needed replacing. As a result this has recently led to a recasting of these Directives into a simplified set of legislation. The package separates aspects of food hygiene from animal health and official control issues and is commonly referred to the "hygiene package". This came into force on 1st January 2006.

The new hygiene package consists of 5 pieces for legislation. These are EU regulations which mean that they must be applied within the member state and are legally binding. This contrasts to the previous directives which required implementation within each member state through national legislation and allowed for some interpretation of their requirements. Three regulations are of prime concern for bivalve mollusc production;

- **Hygiene 1**. Regulation EC No. 852/2004 on the hygiene of foodstuffs
- **Hygiene 2**. Regulation EC No 853/2004 Laying down specific hygiene rules for food of animal origin.
- **Hygiene 3**. Regulation EC No 854/2004 Laying down specific rules for the organisation of official control on products of animal origin intended for human consumption.

Hygiene 1 sets down the general requirements for all types of food operators and establishes that the principle responsibility for food safety lies with the food business operator (FBO). The regulation introduces the requirements for application of hazard analysis critical control point (HACCP) principles during the production process. However this does not currently apply to primary production. So in the context of bivalve mollusc production HACCP principles must be applied during treatment such as depuration or cooking but not during harvesting. The regulation also establishes requirements for traceability of food products. The regulation further refers to the microbiological criteria for food stuff regulations which are being revised currently.

Hygiene 2 gives the requirements for foods of animal origin for industry. The specific rules for the production of live bivalve molluscs are given in section VII of the annexe to the regulations. **Hygiene 3** concerns the organisation and application of official controls for products of animal origin by competent authorities in member states. Specific details for live bivalve molluscs are given in annexe II to the regulation.

In general the specific parts of the new hygiene regulations relating to bivalve shellfish production are similar to those contained in the EU directive 91/492.

The first line of controls rely on the monitoring of shellfish harvesting areas for a faecal indicator bacteria, namely *Escherichia coli*, using a standardised method (ISO/TS 16649) to indicate the extent of sewage contamination in the harvesting area. The sanitary quality of the harvesting area determines the level of treatment that the shellfish must undergo before being placed on the market (Table1.).

Table 1. *E. coli* Levels determining the classification of Bivalve Molluscan Shellfish harvest areas.

Category	Microbiological Standard	Treatment required
Class A	<230 *E. coli* per 100g of flesh and intravalvular fluid	may go direct for human consumption
Class B	<4,600 *E. coli* per 100g of flesh and intravalvular fluid	must be depurated, heat treated or relayed to meet class A requirements
Class C	<46,000 *E. coli* per 100g of flesh and intravalvular fluid	relay for 2 months to meet class A or B requirements May also be heat treated
Prohibited	>60,000 faecal coliforms	harvesting prohibited

An additional measure within the new hygiene regulations (hygiene 3) is that it introduces a provision for ensuring that where the competent authority decides in principle to classify a production or relay area, it must undertake a sanitary survey of that area and that the results from the sanitary survey must be used to select appropriate sampling points when establishing an ongoing sampling programme. This additional requirement provides a scientific basis for developing a monitoring programme in member states. In order to facilitate a consistent approach to developing microbiological monitoring programme across Europe a good practice guide was published in 2006. The guide was established by a working group, commissioned by DGSanco, with the European Community reference laboratory (CRL) taking scientific leadership of the working group. The document includes guidance on all aspects of microbiology monitoring for the purposes of classification of shellfish harvesting areas including conducting sanitary surveys. The document is available on the CRL website **WWW.CRLCEFAS.ORG/**

Under the EU regulations all bivalve molluscan shellfish must meet an end-product standard of <230 *E. coli* $100g^{-1}$ and absence of salmonella in 25g of shellfish flesh either when harvested or following the appropriate treatment. It is a responsibility on all bivalve shellfish producers to undertake regular monitoring of their product entering the market to ensure compliance with this

standard. This should involve a degree of end-product testing for *E. coli* and Salmonella spp. by shellfish producers.

CURRENT APPROVED TREATMENT PROCESSES

Depending on the level of sewage contamination as judged from the *E. coli* levels (Table 1.) certain levels of post harvest treatment are required. These treatments vary in their ability to produce shellfish free from viruses.

In order to render non-category A shellfish fit for consumption three principle, post harvest, treatment processes exist. Firstly heat treatment (cooking) by an approved process can be used to destroy pathogens before consumption. Secondly, relaying shellfish harvested from polluted areas in microbiologically clean environments can be used to allow shellfish to cleanse themselves of microorganisms. Both heat treatment and relaying are deemed suitable for treating shellfish from both category B and C harvesting areas. Alternatively contaminated shellfish can be purified by placing them in tanks of clean seawater to allow them to continue the natural filter-feeding process and purge themselves of sewage microorganisms. This process is called depuration and is only considered acceptable for use with Category B shellfish. As many shellfish species are traditionally eaten raw or only lightly cooked and because of difficulty in finding pristine marine environments for relaying, depuration is often the preferred treatment option and is practised extensively throughout Europe.

Depuration

Depuration is widely practiced throughout Europe. Depuration has been shown to be effective at removing bacteria from shellfish and category B levels of bacteria (up to 4,600 *E. coli* MPN $100g^{-1}$) and much higher are consistently eliminated within 24-48 hours during depuration in well designed and operated depuration tanks (Dore *et al*, 2000). This efficient elimination of bacteria during the depuration process may be responsible for the extremely low incidence of bacterial illness associated with shellfish consumption (Lees, 2000) In contrast, viral elimination during depuration is known to be relatively ineffective and numerous outbreaks of viral illness have been associated with the consumption of depurated shellfish (Murphy *et al*., 1979; Chalmers and McMillan, 1995; Perret and Kudesia, 1995). Numerous laboratory studies have demonstrated either directly or indirectly using bacteriophages as a virus model the persistence of humans viruses compared with sewage derived bacteria during depuration (reviewed in Lees, 2000). As there is very little bacterial illness associated with depurated shellfish and because depuration often does not remove the viral risk, it is often argued that there is little advantage in depurating shellfish under current procedures. However it should be remembered that bacterial illness can still occur after the consumption of sewage-contaminated shellfish. Such outbreaks are still observed where shellfish have been illegally harvested and not treated correctly, demonstrating the potential dangers of relaxing depuration standards. Similarly whilst viruses are not always eliminated completely by depuration, viral levels are reduced during depuration (Henshilwood *et al*, 2003) and the process undoubtedly helps to reduce the viral risk associated

with contaminated shellfish. However the extent to which depuration reduces the risk of viral illness from shellfish consumption remains unclear.

Temperature has been shown to have a major effect on the rate of virus depuration from shellfish. In general increases in water temperature have been shown to increase the rate of viral depuration. Studies by Power and Collins (1991) typically demonstrated that mussels (*Mytilus edulis*) reduced bacteriophages by >99% at 16.5°C after 52 hours depuration compared with only 57% at 5.5°C after the same period of time. Subsequent work using both FRNA bateriophage models and human pathogenic viruses further demonstrated increased virus reductions with increasing temperatures (Dore *et al*, 1995; Henshilwood *et al*, 2003). This work led to suggestions that elevated temperatures and extended treatment times could be used to improve the depuration procedure and that virus standards could be introduced to monitor it's effectiveness. However in this idea has not been developed further. In consultation with stakeholders it was considered that the effect of placing shellfish into tanks of seawater at temperatures well above those of the beds from which they were harvested would affect the shellfish quality and may induce spawning. Additionally, elevating seawater temperature for extended periods was not considered to be economically viable. Consequently the use of elevated temperatures for extended periods of time has not been adopted and there are no plans to do so. Currently no standardised time and temperature regimes exist for depuration within Europe and neither are specified in EU regulations.

Relaying

During relaying (reviewed in Richards, 1987), contaminated shellfish are moved to microbiologically clean environments to remove microbial contaminants in the natural setting. It is an alternative to depuration whereby shellfish can be purified for longer than during depuration but in a less controlled manner. Relaying is considered to be suitable for shellfish from category B and C classified harvesting areas. Relaying is relatively rarely practiced in Europe because of a lack of water of suitable quality or the economic impact of using the treatment. Current EU regulations require that conditions for relaying must ensure optimal conditions for purification and include requirements on the length of time relaying must be carried out for.

Limited data is available on the effectiveness of relaying for virus removal from shellfish. However work has been conducted that indicates that virus removal may be eliminated following relaying for 4-6 weeks combined with subsequent depuration (Dore *et al*, 1998). Water temperature and quality appear to be critical parameters in determining the effectiveness of virus removal. There is a clear need to more closely define criteria for successful relaying.

Heat Treatment (Cooking)

Heat treatment by an approved process can be used to treat category B and C shellfish.

The currently approved treatment methods are:

- sterilisation in hermetically sealed containers
- immersion in boiling water for the period required to raise the internal temperature of the mollusc flesh to not less than 90 °C and maintenance of this minimum temperature for a period of not less than 90 seconds,
- cooking for three to five minutes in an enclosed space where the temperature is between 120 and 160 °C and the pressure is between 2 and 5 kg/cm^2, followed by shelling and freezing of the flesh to a core temperature of -20 °C.
- steaming under pressure in an enclosed space satisfying the requirements relating to cooking time and the internal temperature of the mollusc flesh mentioned under (i) and for which a validated methodology in the framework of an own-checks programme ensures the uniform distribution of heat.

Such cooking is not appropriate for many shellfish such as oysters and clams because these are traditionally consumed raw or only lightly cooked. All these processes, if performed correctly are effective at removing all risk of viral infection from such shellfish.

VIRUS ILLNESS ASSOCIATED WITH CONSUMPTION OF TREATED BIVALVE SHELLFISH

The implementation of legislative controls and the application of approved treatment processes have been successful in virtually eliminating the bacterial risk associated with the consumption of bivalve molluscs within the EU (Lees, 2000). However there remains a significant risk to consumers of viral illness from sewage-contaminated shellfish. Numerous outbreaks of viral illness have occurred following the consumption of treated shellfish (apart from heat treatment) which are fully compliant with the legislative requirements and meet the end-product standard of <230 *E. coli* 100g^{-1}. (Murphy *et al*., 1979; Chalmers and McMillan, 1995; Perret and Kudesia, 1995). Illnesses principally associated with treated shellfish are gastroenteritis caused by NoVs and infectious hepatitis caused by HAV (appendix 1.). A Lack of standardised test methods for detecting human pathogenic viruses in shellfish has been a major barrier to developing and implementing improved controls. However in recent years major technological breakthroughs have been made in this area.

CURRENT AND EMERGING TECHNOLOGY FOR DETECTING VIRUSES IN SHELLFISH

Despite numerous attempts to find a culture system, it remains impossible to detect NoVs in tissue culture. Therefore detection methods in shellfish have relied on the use of molecular techniques and in particular the use of the amplification procedure polymerase chain reaction (PCR). Whilst it is possible to culture HAV it is difficult and cumbersome to achieve from shellfish samples and the use of PCR techniques is also the option of choice for detection.

The PCR reaction is used to amplify a specific sequence of DNA using two short DNA sequences (primers). Figure 1 illustrates the principles involved in the PCR test. Each primer is complementary to one end of the DNA target sequence. In the first cycle the target DNA is separated into two strands by heating to high temperatures. The temperature is reduced to allow the primers to bind to the template DNA. After this step the temperature is increased to allow the enzyme DNA polymerase to make a copy of the target DNA. Two copies of the target DNA are generated after the first cycle. All of these temperature steps are repeated again in the second PCR cycle in which a further two copies of the target are synthesized from one original. A typical PCR reaction has 20-50 cycles, and if the PCR is 100 % efficient, theoretically one target molecule would become 2^n after n cycles. The resulting amplified PCR product can then be visualized as a band on an agarose gel following electrophoresis treatment.

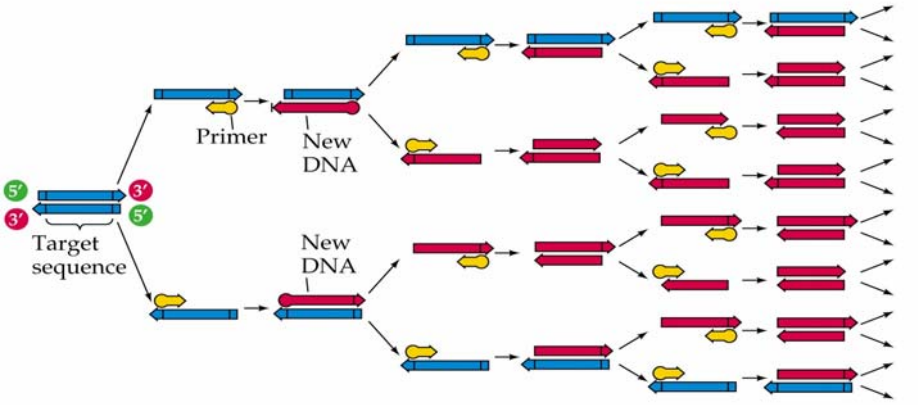

Figure 1. Diagrammatic representation of the PCR

PCR methods for detection of viruses in shellfish have a number of technical limitations which have acted as a barrier to their routine adoption for use during monitoring of shellfisheries for sanitary quality and for assessing virus risk in shellfish. These limitations are that the assays;

- Only allow presence or absence testing (not quantitative)
- Require time consuming confirmation of presumptive positive results
- Are poorly adapted to allow standardisation of the assays

More recently, the development of technically improved PCR procedures, namely real-time PCR methods, has addressed some of these technical deficiencies.

Real-time PCR operates on a similar principle as conventional PCR, as described above, however the PCR reaction can be observed as it is actually happening (i.e. in real time) using the sophisticated real-time PCR instrumentation. The chemistry applied in the real-time PCR assays called TaqMan® (also known as “fluorogenic 5’ nuclease activity”). In TaqMan® PCR an additional short DNA sequence (the probe) binds internally of the two primers. Two fluorescent labels are attached to either end of the probe

sequence. The chemistry of the primer/probe arrangement is such that as the quantity of amplified product increases, fluorescent signal also increases proportionately. The real-time PCR machine used for the TaqMan® PCR detects and records the increasing fluorescence in each sample throughout the cycles. Other systems for real-time PCR are also available but the TaqMan® approach appears to be the most commonly adopted for shellfish.

The results from the real-time PCR assays for NV are achieved more rapidly than the conventional nested PCR assays. There is no need for agarose gel electrophoresis in order to visualise the PCR products as the machine is constantly tracking the amplification of the specific PCR product, which can be viewed on screen (Fig. 2). In addition there is no requirement for confirmation of the assay result with nucleotide sequencing as the inclusion of the strain specific probe confirms the presence and identity of the two different genogroups. The primers and probes for the real-time PCR assays for NV are designed to target the open reading frame (ORF) 1 and ORF2 junction and are listed in table 2.

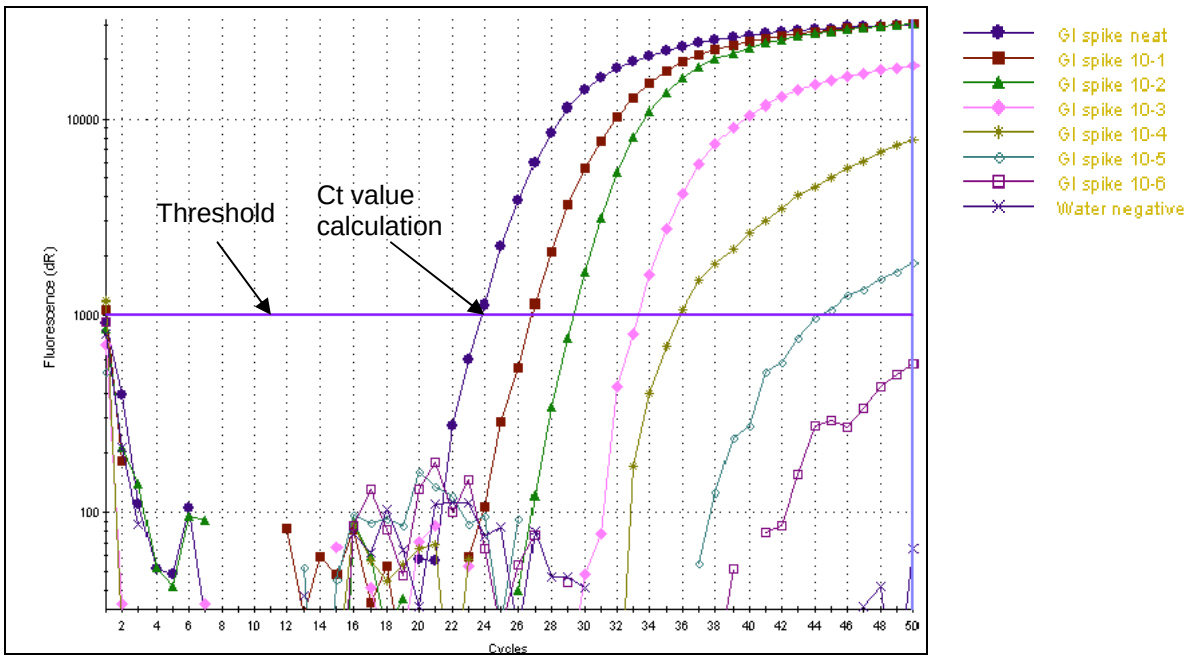

Figure 2. Example amplification plots for NV in shellfish using real-time PCR. Oyster sample was spike with NV faecal material after which viral RNA was isolated and reverse transcribed to cDNA. cDNA was serially diluted and run as template on the NV GI real-time PCR assay.

For the first time, as a result of these improved assays, the prospect of using viral monitoring of shellfisheries to determine the virus risk is being considered internationally.

Standardisation of molecular methods for NV and HAV

Currently molecular methods for detecting viruses in shellfish are poorly standardised for routine use and currently preclude the introduction of regulatory standards. The real-time PCR methods are however more robust than conventional PCR methods and lend themselves more readily to standardisation. The quantitative nature of the real-time PCR assays the use

appropriate internal process controls to ensure the integrity of results. This opportunity to produce standardised methods has been recognised by the scientific community and there are clear efforts on a number of fronts within Europe to standardise the new methods. These are detailed below.

CEN working group – "Detection of viruses in food"

CEN, the European Committee for Standardisation, was founded in 1961 by the national standards bodies in the EEC and EFTA countries. In 2004 the working group (CEN/TC 275/WG 6/TAG 4) on "Detection of viruses in food" was established with the aim of developing a horizontal method for NV and HAV in a range of foodstuffs, including shellfish. Thus far a consensus has been reached on a number of important aspects of method standardisation: use of real-time PCR (probe based) format, the extraction method for isolation of viral RNA from shellfish and use of controls (positive/negative RNA extraction control and internal positive control for PCR inhibition). The next phase due commence in 2007 is to undertake validation trials of the selected finalised method among participating laboratories.

Community reference laboratory (CRL) ring trials

The Centre for Environment, Fisheries and Aquaculture Science (CEFAS) at Weymouth, UK is designated as the Community reference laboratory (CRL) for monitoring the viral and bacteriological contamination of bivalve molluscs. As part of its duties the laboratory is responsible for organising comparative testing by the national reference laboratories designated in each member state. As a result the CRL have established a virus proficiency testing scheme. In 2004/05, samples of variously high, moderate and low titre tissue culture grown HAV and faecal material containing norovirus either singularly or in combination were distributed to NRLs. Each lab uses their own in house methods and the data generated from the scheme has enabled assessments of method specific and laboratory performance, which contributes towards method standardisation. In 2005/06 a distribution was undertaken comprising of Pacific Oysters bioaccumulated with HAV and NV GI and II. Further ring trials are ongoing.

SEAFOODplus – REFHEPA project

This project entitled "Development of standard reference methods for hepatitis A virus and norovirus in bivalve molluscan shellfish", aims to develop and validate standardised PCR assays for the detection of HAV and NV to the point at which they can be used successfully in a routine diagnostic context. The group is working closely with the CEN working group on "Detection of viruses in food", with a particular reference to and emphasis on shellfish. The next step of the project will be to undertake validation studies of proposed virus methods across a number of selected laboratories.

Interpretation of virus positive results obtained using real-time PCR procedures

Limited data is available on the presence of norovirus in shellfish. Studies to date using the new real-time PCR procedures have tended to concentrate on more highly polluted category B areas or problematic harvest areas that have been associated with illness or pollution events. As a result the limited data

generated by these studies should be seen in the context of this potential bias and interpretation of results needs careful consideration. In general a number of unpublished studies by European laboratories have shown that NoVs are commonly found in shellfish from harvesting areas but often only to low levels as judged by the Ct values obtained and the number of positive replicates from those tested. In some studies shellfish areas are consistently positive for Norovirus during the winter months when the chance of viral infection from shellfish consumption is at its greatest. While virus was detected in all samples at some sites no reports of illness were recorded despite widespread harvesting of shellfish. However, it is well known that the incidence of norovirus outbreaks associated with food is vastly underestimated and it cannot be ruled out that some of the shellfish from the harvest areas did cause illness.

Clearly the detection of low levels of virus in itself may not equate directly to risk of illness in individual samples. A number of issues may be of importance in distinguishing shellfish containing norovirus and causing illness from those containing norovirus and do not cause illness. These may include virus titre (levels), norovirus strain and uncertainty as to whether virus positive results associated with the PCR are viable and therefore infectious. The doubt associated with the risk posed by virus positive shellfish by real-time causes a significant challenge for risk managers going forward. However It is apparent that definitive interpretation of norovirus results is problematic and requires further investigation. Using standardised virus detection methods to determine the factors which may affect risk of virus infection i.e. titre, infectivity and virus strain.

There is less confusion over the situation with HAV where it would be reasonable to assume, given the severity of the disease and the seemingly less frequent occurrence in European shellfish, that detection of any level of virus should be considered to represent a significant risk to the consumer.

RISK MANAGEMENT OF SHELLFISH HARVESTING AREAS

While it may appear attractive to rely on batch testing of food products for specific pathogens to determine their compliance with legislative standards and assess the potential risk associated with that product, in general this approach is not effective if practiced in isolation. There are number of reasons for this;

- Pathogens are not usually distributed evenly within batches of food products
- Pathogen presence in food is often a transient event occurring in specific batches which requires testing of every batch to ensure detection of such events
- Negative results give no information on the likelihood of future batches being positive.
- Often a range of pathogens may potentially be present in food product and testing for one, will generally give no information on the likely

presence of another. (e.g. a NoV negative result gives no information on the presence of HAV in shellfish)

As result it is generally accepted that control and validation of the whole food production process is a more appropriate way of controlling public health risks. Generally this is developed using the concept of Hazard Analysis and Critical Control Point (HACCP). This is based on

- Conducting a hazard analysis
- Identifying critical control points (CCPs)
- Establishing critical limits
- Establishing a system to monitor control of the critical control points
- Establish a corrective action plan when critical control points are out of control.
- Establish procedures to verify that the HACCP system is working effectively.

In general the use of the HACCP procedures may be considered to have been poorly applied to the production of shellfish in Europe. In particular the reliance on compliance with microbiological standards to validate the the HACCP approach for shellfish have been shown to be flawed.

Although there is currently no legal driver there is now a growing move towards the developing HACCP type approaches to produce risk management procedures for reducing the risk of viral illness associated with shellfish.

An approach to achieving this is to;

- identify pollution sources impacting on shellfish in a bay and assess there contribution to contamination of the shellfish.
- identify conditions under which viral contamination occurs in the shellfisheries.
- identify appropriate intervention strategies to reduce the risk of illness

Identification of sources polluting and there contribution to towards contamination requires a sanitary survey. In the new legislative controls introduced on January 1st 2006 there is a requirement to undertake sanitary surveys of newly designated shellfish harvesting areas. However this requirement is only intended to inform the competent authority on the position of sampling location for the classification monitoring programme. The recently published Good Practice Guide gives details on the principles of conducting a sanitary survey. In addition the CRL has run training events on conducting sanitary surveys. Such sanitary surveys could form the basis for initial steps in introducing a risk-based management approach to controlling the risk in shellfisheries.

There are a number of steps which will form part of the sanitary survey. These include;

- Characterisation of the fishery including, Location, size, species, harvesting methods
- Identification of pollution sources including continuous sewage discharges point sources of pollution such as sewage treatment plants, and diffuse sources of pollution such agricultural run-off. As much information on the source should be gathered as possible
- Gathering and assessing hydrographic and hydrodynamic information to determine the characteristics of the circulation of contamination.
- Microbiological surveys may also be performed to assess the extent of contamination.

Of particular importance when considering the implementation of risk management procedures in shellfisheries is being able to identify conditions which result in viral contamination of shellfisheries. Once these conditions are identified in a bay it is then possible to identify indicators of the conditions and to use the triggers to implement early warning systems that can react to the threat in real time. This is opposed to microbiological monitoring which occurs retrospectively. Conditions that may lead to increased risk of viral contamination include;

- High rainfall levels causing sewage treatment plants to overflow releasing untreated sewage into the harvesting area
- Contamination events – failure of sewage treatment plants.
- High levels of infections in the community leading to increased viral shedding into the environment.

Work in identifying conditions associated with viral contamination and associated indicators that can be used as triggers to identify increased risks is being developed in the REDRISK project as part of SEAFOODplus. The use of the newly developed real-time procedures for detecting viruses has assisted in this process.

Results from the REDRISK project and the use of new viral testing procedures clearly demonstrate that it is now technically feasible, through risk identification (sanitary surveys) and viral monitoring, to identify and predict when viral contamination occurs in shellfisheries. However it is equally clear that the challenges in developing risk management procedures for shellfish harvesting areas remain considerable. The identification of indicators of conditions responsible for viral contamination that could be used in real-time to trigger intervention measures, although now feasible, is complex and site specific. Thus in depth knowledge and assessment of individual areas is required before site-specific triggers for intervention can developed. This has clear resource implication and presents challenges for risk managers and competent authorities throughout Europe. Further development of improved treatment procedures and intervention strategies are also required.

REFERENCES

Chalmers, J. W. T., and J. H. McMillan. 1995. An outbreak of viral gastroenteritis with adequately prepared oysters. Epidemiol. Infect. 115: 163-167.

Doré W.J. and D.N. Lees 1995. Behavior of Escherichia coli and Male-Specific Bacteriophage in Environmentally Contaminated Bivalve Molluscs before and after Depuration. Applied and Environ. Microbiol. 61: 2830 - 2834.

Dore, W.J., Henshilwood, K. and Lees, D.N. (1998) The development of management strategies for control of virological quality in oysters. *Water Science and Technology* 38, 29-35.

Dore, W.J., Henshilwood, K. and Lees, D.N. 2000. Evaluation of F-Specific RNA bacteriophage as a candidate human enteric virus indicator for bivalve molluscan shellfish. Applied. Environ. Microbiol. 66:1280-1285

European Communities 2004. Regulation (EC) No 882/2004 of the European Parliament and of the Council of 29 April 2004 on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules. *Off. J. Eur. Communities* L 165, 30.4.04 : 1-141.

European Communities 2004. Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for food of animal origin. *Off. J. Eur. Communities* L 226, 25.6.04 : 22-82.

European Communities 2004. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. *Off. J. Eur. Communities* L 226, 25.6.04 : 83-127.

Fleet, G.H. 1978. Oyster depuration - a review. Food Technol. Aust. 30:444-454.

Henshilwood, K., Dore, W., Anderson, S. and Lees, D. (2003) The Development of a quantitative assay for the detection of Norwalk like virus and its application to depuration. Proceedings of the 4th International Conference on Molluscan Shellfish Safety. June 4-8 2002, Santiago de Compostela, Spain,

ISO/TS 16649-3:2005: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli - Part 3: Most probable number technique using 5-bromo-4-chloro-3-indolyl-beta-D-glucuronide

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

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

Perret, K., and G. Kudesia. 1995. Gastroenteritis associated with oysters. Public health Laboratory Sevice. Communicable Disease Report 5:R153-154.

Power, U.F. and J.K. Collins. 1989. Elimination of coliphages and *Escherichia coli* from mussels during depuration under varying conditions of temperature, salinity and food availability. J. Food Prot. 53: 208-212.

Richards, G.P. 1987. Microbial Purification of shellfish: A review of depuration and relaying. J. Food Prot. 51:218-251.

GLOSSARY

Bivalve Molluscs – Any marine mollusc of the class *Pelecypoda* (formerly *Bivalvia* or *Lamellibranchia*), having a laterally compressed body, a shellconsisting of two hinged valve, and gills for respiration. The groupincludes clams, cockles, oysters, and mussels. In European food legislation the term also covers echinoderms, tunicates and marine gastropods.

Competent Authority – the central authority of an EU member state competent for the organisation of official controls.

Deoxyribonucleic Acid (DNA) – A nucleic acid that carries the genetic information in the cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chains of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or cytosine and guanine.

Depuration – Purification of bivalve Molluscan shellfish by placing in tanks of clean seawater and allowing them to purge themselves of microbiological contaminants through the filter feeding process.

***Escherichia coli* -** A species of bacterium that is a member of the faecal coliform group. It is more specifically associated with the intestines of warm-blooded animals and birds than other members of the faecal coliform group. *E. coli* is determined in European the reference method on the basis of the possession of β-glucuronidase activity.

Heat treatment by an approved method – Heat treatment processes that have been found to be effective to prevent infectious illness associated with shellfish consumption and have been I specifically identified in EU regulations. This does not cover home cooking which is not controlled and may not be effective at destroying viruses.

Hepatitis A Virus – is a small (27 nm) RNA viruses transmitted by the faecal oral route. Most infections are asymptomatic in the young but may cause fever and inflammation of the liver leading to jaundice in susceptible adults**.**

Norovirus – is a small (27-32nm) RNA virus belonging to the calicivirus family. They cause gastroenteritis and are spread by the faecal oral route. They are responsible for the majority of non-bacterial gastroenteritis outbreaks in the community. Noroviruses were previously called small round structured viruses (SRSVs) and Norwalk-like virus (NLVs) and are commonly referred to as the winter vomiting bug because of there seasonal distribution.

Polymerase Chain Reaction (PCR) - A technique for amplifying DNA sequences in vitro by separating the DNA into two strands and incubating it with oligonucleotide primers and DNA polymerase. It can amplify a specific sequence of DNA by as many as one billion times.

Relaying – is the movement of shellfish from relatively contaminated harvesting areas to new areas which are considered to be microbiologically "clean" where they are allowed to purge themselves of contaminants through their natural filter feeding process.

Ribonucleic Acid (RNA) - A polymeric constituent of all living cells and many viruses, consisting of a long, usually single-stranded chain of alternating phosphate and ribose units with the bases adenine, guanine, cytosine, and uracil bonded to the ribose. The structure and base sequence of RNA are determinants of protein synthesis and the transmission of genetic information.

Sanitary survey – identification of the sources of faecal contamination associated with a shellfish harvesting area and an assessment of the potential impact on the microbiological status of the harvesting area.

Appendix 1. Characteristics of norovirus and hepatitis A virus.

Hepatitis A

Hepatitis A virus (HAV) is classified with the enterovirus group of the Picornaviridae family. HAV is excreted in faeces of infected people and can produce clinical disease by person-to-person spread by the faecal oral route. Susceptible individuals consuming contaminated water or foods are also at risk. Cooked meats and sandwiches, fruits and fruit juices, milk and milk products, vegetables, salads, shellfish, and iced drinks are commonly implicated in outbreaks. Water, shellfish, and salads are the most frequent sources of food-associated infections. Contamination of foods by infected workers in food processing plants and restaurants is common.

Infectious hepatitis caused by HAV is usually a mild illness characterized by sudden onset of fever, malaise, nausea, anorexia, and abdominal discomfort, followed in several days by jaundice. The infectious dose is unknown but is presumed to be 10-100 virus particles. The incubation period for hepatitis A, which varies from 10 to 50 days (mean 30 days), is dependent upon the number of infectious particles consumed. Infection with very few particles results in longer incubation periods. The period of virus shedding extends from early in the incubation period to about a week after the development of jaundice. The greatest danger of spreading the disease to others occurs during the middle of the incubation period, well before the first presentation of symptoms. Many infections with HAV do not result in clinical disease, especially in children. When disease does occur, it is usually mild and recovery is complete in 1-2 weeks. Occasionally, the symptoms are severe and convalescence can take several months. Patients suffer from feeling chronically tired during convalescence, and their inability to work can cause considerable financial loss. Death can occur but is very rare and usually associated with the elderly.

Hepatitis A has a worldwide distribution occurring in both epidemic and sporadic fashions. In developing countries, the incidence of disease in adults is relatively low because of exposure to the virus in childhood. Childhood infection is often asymptomatic and causes lifelong immunity. In such countries most individuals, 18 and older demonstrate immunity to re-infection. However, improving sanitary conditions in developed countries means exposure to HAV during childhood is increasingly rare. Consequently, many adult individuals are not protected through immunity and are susceptible to infection. The increased number of susceptible individuals allows common source epidemics to evolve rapidly.

Norovirus

Norovirus (NV) is one of the four genera of the calicivirus that make up the *Caliciviridae* family. The first human calicivirus to be identified was Norwalk virus in 1972, following an outbreak of virus gastroenteritis at a primary school in Norwalk, Ohio, USA in 1968. Subsequently further morphologically similar,

although antigenically distinct, viruses have been identified as causes of outbreaks of virus gastroenteritis. These viruses have at various times been termed "small round structured viruses" (SRSVs) and “Norwalk Like Viruses” or "classic human calicivirus" and were generally named after the place in which the outbreak occurred such as Southampton, Mexico and Hawaii virus.

Noroviruses cause acute gastroenteritis which is usually a mild self-limiting infection lasting 12 to 24 hours. Symptoms often include diarrhoea, nausea, vomiting and abdominal pain; more rarely reported are fever and headache. Illness is caused by infection of the intestinal mucosa and destruction of absorptive cells at the top of the intestinal villi and follows an incubation period of 24 to 60 hours. Some infections are more severe and hospitalisation of elderly and debilitated individuals has been required on rare occasions. In countries where molecular and serological tests are used for diagnostic purposes NVs have been identified as the major cause of epidemic gastroenteritis. This includes the UK, The Netherlands, Japan, and the United States. It is now apparent that many of the non-bacterial outbreaks of gastroenteritis in the 1970s through to the early 90s to which an aetiological agent could not be ascribed, were probably a result of NV infections.

Transmission of NVs is via the faecal-oral route either directly or via contaminated food or water. The virus is highly infectious requiring a low infectious dose, maybe <10 virus particles. Person-to-person transmission is common especially in closed communities and large outbreaks of NV induced gastroenteritis have been reported in hospitals , nursing homes, cruise ships and military settings. Infections in Northern Europe demonstrate a strong seasonal distribution with infections peaking in the winter months (November through to March). In fact this distribution is so pronounced that acute gastroenteritis caused by NVs was originally described as winter vomiting disease.

NV contamination of food and water supplies has been shown to be responsible for large numbers of outbreaks. Food contamination can occur at source such as the case with sewage contaminated shellfish or post treatment by ill or asymptomatic food handlers. Patients show prolonged asymptomatic shedding of the virus for up to two weeks.

The immunological response to NV infection is complex and still relatively poorly understood. Infection by NVs has been shown to result in short-term immunity with little cross protection between different strains. However studies have demonstrated that pre-existing antibody levels in volunteers before exposure to NVs do not correlate with resistance to infection. In fact, paradoxically, increased antibody levels appear to correlate with increased risk of infection. It has been hypothesised that detection of antibody levels indicate previous infection without necessarily conferring immunity and suggest the host is susceptible. Conversely it is proposed that absence of antibodies may be indicative of no past infection and a predisposed resistance which is not necessarily due to immune response. Recently it has been suggested that susceptibility and resistance may be associated with an individual’s blood group. Individuals with an O antigen phenotype were more

likely to be infected with NV than individuals with a blood group B antigen. The recent use of immunological assays based recombinant virus like particles in epidemiological studies have demonstrated that the age of antibody acquisition and illness is much lower than previously believed. It appears that infection can occur early in life and repeated re-infection can occur throughout life in susceptible individuals

HAV and NV Bibliography

Berg, D.E., Kohn, M.A., Farley, T.A. and Mcfarland, L.M. (2000) Multi-state outbreaks of acute gastroenteritis traced to fecally-contaminated oysters harvested in louisiana. *J. infect. Dis.* **181** S381-S386.

Caul, E., Sellwood, J., Brown, D.W., Curry, A., Humphrey, T.J., Hutchinson, D.N., Kurtz, J.B., Palmer, S.R., Riordan, T. and Sharp, I. (1993) Outbreaks of gastroenteritis associated with SRSVs. *PHLS Microbiol. Digest* **10** 2-8.

Chadwick, P.R. and McCann, R. (1994) Transmission of a small round structured virus by vomiting during a hospital outbreak of gastroenteritis. *J. Hosp. Infect.* **26** 251-259.

Chalmers, J.W.T. and Mcmillan, J.H. (1995) An outbreak of virus gastroenteritis associated with adequately prepared oysters. *Epidemiology & Infection* **115** 163-167.

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 Shellfish Res* **17** 1633-1635.

Clarke, I. N. and Lambden, P. R. Organization and expression of calicivirus genes. *J. infect. Dis.* **181** S309-S316. 2000.

Cliver,D.O. (1997) Virus transmission via food. *Food Technol.* **51** 71-78.

Dingle, K.E., Lambden, P.R., Caul, E.O. and Clarke, I.N. (1995) Human enteric caliciviridae: the complete genome sequence and expression of virus-like particles from a genetic group II small round structured virus. *J.Gen. Virol.* **76** 2349-2355.

Estes, M.K. and Leparc-Goffart, I. (1999) Norwalk and Related Viruses. In *Encyclopedia of Virology* ed. Granoff,A. and Webster pp. 1035-1041.

Eyles, M.J. (1986) Transmission of viral disease by food: an update. *Food Technol. Australia.* **38**, 239-250.

Fankhauser, R.L., Noel, S., Monroe, S.S., Ando, T. and Glass, R.I. (1998) Molecular epidemiology of "norwalk-like viruses" in outbreaks of gastroenteritis in the united states. *J. Infect. Dis.* **178**, 1571-1578.

Gellert, G.A., Waterman, S.W., Ewert, D., Oshiro, L., Giles, M.P., Monroe, S.S., Gorelkin, L. and Glass, R.I. (1990) An outbreak of acute gastroenteritis caused by a small round structured virus in a geriatric convalescent facility. *Infect. Control. Hosp. Epidemiol.* **11** 459-464.

Gillespie, I.A., Adak, G.A., O'Brien, S.J., Brett, M.M. and Bolton, F.J. (2001) General outbreaks of infectious intestinal disease associated with fish and shellfish, England and Wales, 1992-1999. *Comm. Dis. and Pub. Health* **4** 117-123.

Graham, D.Y., Jiang, X., Tanaka, T., Opekun, A.R., Madore, H.P. and Estes, M.K. (1994) Norwalk virus infection of volunteers: New insights based on improved assays. *J. Infect. Dis.* **170** 34-43.

Halliday, M. L., Kang, L.-Y., Zhou, T.-K., Hu, M.-D., Pan, Q.-C., Fu, T.-Y., Huang, Y.-S. and Hu, S.-L. (1991) An epidemic of hepatitis a attributable to the ingestion of raw clams in shanghai china. *J. Infect. Dis.* **164** 852-859.

Hutson, A.M.; Atmar, R.L.; Graham, D.Y.; Estes, M.K. (2002) Norwalk virus infection and disease is associated with ABO histo-blood group type J. Infec. Dis. **185** 1335-1337

Hyams, K.C., Bourgeois, A.L., Merrell, B.R., Rozmajzl, P., Escamilla, J., Thornton, S.A., Wasserman, G.M., Burke, A., and Echeverria, P. (1991) Diarrheal disease during operation desert shield. *N. Engl. J. Med.* **325**, 1423-1428.

Inouye, S., Yamashita, K., Yamadera, S., Yoshikawa, M., Kato, N. and Okabe, N. (2000) Surveillance of viral gastroenteritis in Japan: Pediatric cases and outbreak incidents. *J. infect. Dis.* **181** S270-S274. 2000.

Kapician, A.Z., Wyatt, R.G., Dolin, R., Thornhill, T.S., Kalica, A.R. and Chanock, R.M. (1972) Visualisation by immune electron microscopy of a 27nm particle associated with acute infectious nonbacterial gastroenteritis. *J. Virol.* **10** 1075-1081.

Khan, A.S., Pon, E., Monroe, S.S., Jiang, X., Estes, M.K., Iskander, J., Chapman, L.E., Schonberger, L.B. and Glass, R.I. (1992) Norwalk-associated gastroenteritis traced to ice exposure aboard a cruise ship in hawaii application of molecular based assays. Abstr. *Intersci. Conf. Antimicrob. Agents Chemother.* **32** 342.

Koopmans, M., Vinjé, J., deWit, M., Leenen, I., vanderPoel, W. and vanDuynhoven, Y. (2000) Molecular epidemiology of human enteric caliciviruses in The Netherlands. *J. infect. Dis.* **181** S262-S269

Lambden, P.R., Caul, E.O., Ashley, C.R. and Clarke, I.N. (1993) Sequence and genome organization of a human small round- structured norwalk-like virus. *Science* **259** 516-519.

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

Matsui, S.M. and Greenberg, H.B. Immunity to calicivirus infection. *J. infect. Dis.* **181** S331-S335. 2000.

Millard, J., Appleton, H. and Parry, J.V. (1987) Studies on heat inactivation of hepatitis a virus with special reference to shellfish. *Epidemiol. Infect.* **98**, 397-414.

Mounts, A.W., Ando, T., Koopmans, M., Bresee, J.S., Noel, J. and Glass, R.I. (2000) Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J. infect. Dis.* **181**, S284-S287. 2000.

Newsholme, A. (1896) The spread of enteric fever by means of sewage polluted shellfish. *R. J. San. Inst.* **3** 389-413.

Patterson, T., Hutchings, P. and Palmer, S. (1993) Outbreak of SRSV gastroenteritis at an international conference traced to food handled by a post-symptomatic caterer. *Epidemiol. Infect.* **111** 157-162.

Patterson, W., Haswell, P., Fryers, P.T. and Green, J. (1997) Outbreak of small round structured virus gastroenteritis arose after kitchen assistant vomited. *Comm. Dis. Rep.* **7** 101-103.

Perrett, K. and Kudesia, G. (1995) Gastroenteritis associated with oysters. *Comm. Disease Rep.* **5** 141-156.

Pontefract, 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.

Pringle, C.R. (1998) Virus taxonomy - san diego 1998. *Arch. Virol.* **143** 1449-1459.

Richards, G.P. (1985) Shellfish-associated enteric virus illness in the United States. *Estuaries.* **8** 94-96.

Roos, R. (1956) Hepatitis epidemic conveyed by oysters. *Sven Lakartidningen* **53** 989-1003.

Schaub, S.A. and Oshiro, R.K. (2000) Public health concerns about caliciviruses as waterborne contaminants. *J. infect. Dis.* **181** S374-S380.

Sharp, T.W., Thornton, S.A., Wallace, M.R., Defraites, R.E., Sanchez, J.L., Batchelor, R.A., Rozmajzl, P.J., Hanson, R.K., Echeverria, P., Kapikian, A.Z., Xiang, X.J., Estes, M.K. and Burans, J.P. (1995) Diarrheal disease among military personnel during operation restore hope, somalia, 1992-1993. *Am. J. Tropical Med. & Hygiene* **52** 188-193.

Sugieda, M., Nakajima, K. and Nakajima, S. (1996) Outbreaks of norwalk-like virus-associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. *Epidemiol. Infect.* **116** 339-346.

Taylor, M.B., Schildhauser, C.I., Parker, S., Grabow, W.O.K., Jiang, X., Estes, M.K. and Cubitt, W.D. (1993) Two successive outbreaks of srsv-associated gastroenteritis in south africa. *J. Med. Virol.* **41** 18-23.