

ATTACHMENT 6**RISK PROFILE OF *NOROVIRUS* IN BIVALVE MOLLUSCAN SHELLFISH
(Netherlands)****INTRODUCTION**

During the 37th session of the Codex Committee on Food Hygiene (CCFH) it was agreed to place five proposals for new work areas into the Committee's work management system (Report of the 37th Session of CCFH, ALINORM 05/28/13, paragraph 168). The Netherlands was appointed to prepare a written proposal for one of the items, that is, viruses in food.

The ad hoc Working Group for the Establishment of CCFH Work Priorities (ad hoc Working Group) recommended to pursue work on a risk profile on viruses in food and focus early work regarding viruses in food on viruses in seafood in general with emphasis on bivalve molluscs (ALINORM 05/28/13, paragraph 192).

This view is fully adopted by the authors of the present document. More than that, it was deemed necessary to restrict the approach to norovirus in bivalve molluscs at this point, as norovirus infections must be considered an emerging infectious disease with contaminated bivalve molluscs playing a major role in food borne transmission. Other viral infections with regard to bivalve molluscs, particularly hepatitis A, can be addressed in a different stage.

To our opinion the entire issue of food borne viral infections is too diverse to be addressed as a single topic, as transmission routes, product matrices and disorders differ greatly. Because of the complexity of the matter we would strongly suggest to focus on viral agent/product combinations, e.g. noroviruses in shellfish or fresh berries, hepatitis A virus in shellfish or fresh berries, rather than on "viruses in food" in general.

BACKGROUND

Noroviruses (NoV) are formerly known as 'small-round-structured-virus' or 'Norwalk-like virus' (NLV) and belong to the family Caliciviridae. Noroviruses have been associated with gastro-enteritis with an acute onset of nausea, vomiting, abdominal cramps, and diarrhea as prominent symptoms. In adults, projectile vomiting frequently occurs. Constitutional symptoms such as low-grade fever, headache, chills, and myalgia are frequently reported. The illness generally is considered mild and self-limiting, with symptoms lasting on average 12-60 hours (1,2). Besides being the cause of large (institutional) outbreaks, recent data suggest that norovirus are among the most common causes of sporadic gastroenteritis. People from all age groups are affected (3).

Although this highly contagious virus is readily transmitted from person-to-person, noroviruses have also emerged as a food-borne virus that is likely due to an increased awareness combined with improved diagnostic assays. In risk factor analysis, using data collected with questionnaires during a community-based study, it was estimated that 12-17% of the norovirus infections in the Netherlands are likely to be food-related, which estimates the incidence of food-borne norovirus disease in the same range as for *Salmonella* and *Campylobacter* (4). Virtually any food may be implicated in norovirus transmission, but bivalve molluscan shellfish present a particularly high risk because of their ability to concentrate viruses from contaminated waters.

SCOPE AND RATIONALE

The first linkage of viruses with shellfish-borne gastroenteritis was made in 1976/1977 in the UK (5, 6). Since then enteric viruses causing gastroenteritis have been epidemiologically linked to outbreaks of shellfish-vectored illness on numerous occasions and in numerous countries (7). The rationale to focus on noroviruses in shellfish is based on the following

Shellfish can act as a vehicle for transmission of noroviruses.

Microbiological quality control criteria are not sufficiently validated to indicate presence or absence of viral contamination

Shellfish harvested from contaminated areas may contain a cocktail of viruses and the simultaneous infection of patients may lead to the generation of recombinant norovirus strains. New recombinants may be more virulent than the known ones and may cause a sudden rise in number of outbreaks after introduction into a population as was observed in the winter of 2000/2001 (4).

There is a substantial global trade in bivalve molluscan shellfish, which may lead to spreading of new viruses.

Noroviruses serve as a model for other enteric viruses, like hepatitis A virus, hepatitis E virus and the enteroviruses. By filter feeding, bivalve molluscan shellfish may concentrate these enteric viruses as well. Due to the longer incubation periods and high rate of asymptomatic infections, illness caused by these enteric viruses may be more difficult to relate to consumption of shellfish. The detection of norovirus in shellfish may therefore strongly indicate that also other enteric pathogens, that may be less easily detected or diagnosed, are present in food too.

For these reasons appropriate strategies to reduce this documented risks should be developed.

PATHOGEN-FOOD COMMODITY COMBINATIONS OF CONCERN

Pathogen of concern: *Norovirus*

Description of the food or food product and/or condition of its use with which problems (foodborne illness, trade restrictions) due to this hazard have been associated

The bivalve molluscan shellfish, not the finfish or non-filter feeding shellfish, feed by filtering small particles from surrounding water. In this process the bivalve mollusks may concentrate and retain human pathogens derived from sewage contamination. The presence of noroviruses in naturally contaminated shellfish from polluted harvesting areas, or in shellfish associated with disease outbreaks, has been documented in varying percentages depending on the sanitation categories of the harvesting areas (7). The hazards posed by bioaccumulation are compounded by the traditional consumption of certain shellfish species (such as oysters) raw or only lightly cooked (mussels and clams), and by consumption of the whole animal, including the viscera where the human viruses are trapped.

Data on heat inactivation for noroviruses are scarce, because noroviruses can not be grown in cell culture or in an animal model. By comparative analysis, animal caliciviruses and hepatitis A virus have been proposed as model viruses for studies on infectivity. Noroviruses appear to be inactivated by normal cooking processes but are not always inactivated in shellfish given only (minimal) heat treatment as used for the preparation (grilling, stewing or frying) (8). Heating required to open the shells is not necessarily sufficient to inactivate viruses. Norovirus heated to 60 °C for 30 minutes remained infectious for volunteers (9). Raising the internal temperature of the shellfish meat to 90 °C for 1.5 minutes is likely to be sufficient (10). The time required may also depend on the direct environment; for another enteric virus, hepatitis A virus, it was demonstrated that inactivation in mussels was dependent on the recipe applied (11). Also freezing does not inactivate hepatitis A or animal caliciviruses, and is unlikely to affect norovirus infectivity. Frozen foods that did not receive further cooking have been implicated in a number of norovirus outbreaks.

DESCRIPTION OF THE PATHOGEN AND PUBLIC HEALTH PROBLEM

THE PATHOGEN

Noroviruses (NoV), formerly known as 'small-round-structured-virus' or 'Norwalk-like virus' (NLV), belong to the family Caliciviridae. NoV are small, non-enveloped spherical viruses, measuring between 28 and 35 nm in size, that contain a single stranded RNA (ribonucleic) genome of 7.3-7.6 kb. The genome is of positive polarity. It contains coding information for a set of non-structural proteins located at the 5'-end of the genome and for a major structural protein at the 3'-end. Based on sequence data of the capsid and polymerase (POL) areas norovirus found in humans can be divided into three major genetic groups (genogroups). Distinct genotypes have been recognized within each genogroup, of which the number is increasing. Additional noroviruses segregating into a fourth genogroup have been found in cattle.

There is little precise information on the stability, again because no *in vitro* culture systems exist to assess viability. Noroviruses appear to survive on inanimate surfaces and in the environment. Epidemiological evidence from lingering outbreaks that have occurred in hospitals, in residential homes and on cruise ships supports this (9). Noroviruses are more resistant to heat, disinfection and pH changes than are most vegetative bacteria (12). They retain their infectivity after exposure to pH 2.7 for 3 hours at room temperature, but also after refrigeration and freezing. They survive well on inanimate surfaces. They are considered to be resistant to inactivation in the presence of 3.75-6.25 mg chlorine/L, equivalent to 0.5-1.0 mg/L. Noroviruses are inactivated by 10 mg chlorine/L, which is the concentration used to treat a water supply after a contamination incident (9). They may survive for extended periods of time in seawater, especially in the winter months when temperatures are low (7).

THE PUBLIC HEALTH PROBLEM

Studies of community acquired infectious intestinal disease have been done in The Netherlands and in the UK and have demonstrated that viral infections account for a large proportion of community-acquired gastro-enteritis, especially the noroviruses. For the Netherlands (population 16.3 million) it is estimated that >500.000 cases of norovirus illness occur in the community during the study period (1999) (13). Many smaller surveys in limited populations have confirmed the high burden of illness due to noroviruses (4). Data from seroprevalence studies suggest that norovirus infections are found worldwide (14).

Only a few particles are needed to produce illness (15). In outbreaks, the average attack rate is high-typically 45% or more (16). The average incubation period is 12-48 hours after exposure. The illness generally is considered mild and self-limiting, with symptoms lasting on average 12-60 hours (1-3). Asymptomatic infections are also common. A total of 5% of healthy controls were found to shed noroviruses in a community study, as compared with 16% of people with gastroenteritis (13). Similarly, in outbreaks settings, 75% of people with gastroenteritis were found to shed noroviruses compared to 20% of healthy contacts (16).

The incidence of norovirus is highest in young children, but illness also occurs regularly in adults. In addition, the majority of outbreaks of gastroenteritis in institutions such as nursing homes and hospitals is caused by noroviruses (17). The high attack rate in both residents and personnel of such institutions often lead to major understaffing problems during outbreaks. The group of individuals who would be at greatest risk of serious illness and mortality includes young children, the elderly, pregnant women and the immunocompromized (18). Recently evidence was provided for severe clinical features in patients with several underlying diseases, such as cardiovascular disease, renal transplantation and immunosuppressive therapy (19).

Data from a community-based cohort study in the Netherlands were surprising in that 20% of norovirus infected persons reported symptoms for more than two weeks (3). Some long term shedders with long term complaints have been described (20), although no long term sequelae of norovirus infections have

been reported and it remains to be seen if this was a chance observation (21). Sometimes hospitalisation and even parenteral fluid therapy due to severe dehydration are required in norovirus infections. During a norovirus outbreak at an international scout summer camp in the Netherlands up to 18% of affected people were admitted to a local hospital for rehydration (22).

It is known that after experimental infections in volunteers the infected persons may become protected from reinfection, but only for a short period, and only when the challenge virus is closely related to the genotype of the strain that was used for the infection (23, 24). A breakthrough in the field has been the discovery that clear differences in susceptibility have been found between persons with different blood groups and other genetic markers. This is explained by the observation that norovirus particles bind to carbohydrates that are part of the histo-bloodgroup antigens. Further research has shown that the binding properties differ between different genetic variants, thus providing very different patterns of host susceptibility for different norovirus genotypes (25).

The first linkage of viruses with shellfish-borne gastroenteritis was made in 1976/1977 in the UK when cooked cockles were epidemiologically linked to 33 incidents affecting nearly 800 people (5, 6). Subsequently, norovirus-like particles were detected in about 90 % of the clinical samples of nine separate shellfish vectored gastro-enteritis outbreaks (6). Since then enteric viruses causing gastroenteritis have been epidemiologically linked to outbreaks of shellfish-vectored illness on numerous occasions and countries (7). The US FDA risk assessments estimate cases of norovirus gastro-enteritis related to seafood consumption at some 100,000 per year (26). Such estimates for other countries have not been performed or are not readily available in the scientific literature. Data based on outbreak reporting are clearly underestimating the true extent of foodborne transmission.

Progress with clinical PCR assays for noroviruses and other enteric viruses prompted the exploration of the technology to detection of viruses in food and more specifically in seafood. The detection of virus in shellfish associated with outbreaks by PCR techniques have been described (7). Since the year 2000, reports describing the successful linking of cases of viral disease to contaminated food by the demonstration of an identical norovirus sequence in clinical specimens and suspected oysters are becoming available (27-29). The complexity of norovirus detection was described by a French outbreak with oysters (28). Moreover, when multiple norovirus types are present in suspected shellfish, linking may be hard as the predominating norovirus type in the clinical samples may then not be necessarily the same as the norovirus type detected in the suspected shellfish samples.

The amplification of food-borne infections after consumption of shellfish through person-to-person transmission is an issue that needs further consideration. The initial outbreaks will occur in people who ate e.g. oysters, but secondary and tertiary waves of infection may occur, which then are recognized as person-to-person outbreaks. This is exemplified by the following: In the winter of 2000/2001, several outbreaks of norovirus illness developed in 3 countries, associated with imported shellfish. The viruses clearly stood out, because they were of unusual type that had not been observed in most surveys prior to date. Tracking of this virus learned that over 200 outbreaks occurred in 7 countries, following this initial introduction (4).

In addition, in the above example, it was shown that this was a highly unusual virus strain, because it consisted of 4 different recombinant genomes. Recombination can only happen if two viruses infect the same cell at the same time, and mix their genetic material to form a novel virus. It can be postulated that consumption of multiple contaminated oysters constitutes an extra risk for generation of novel norovirus strains (codex 2005)(4). Besides recombination, noroviruses evolve by accumulation on of mutations.” Epidemic waves” in which new variant noroviruses emerged were noticed in 2002 across Europe (30) and in 2004 in a more limited geographic region (31). The mechanisms of evolution of these viruses remains unclear, but given the ample evidence for foodborne transmission, food is likely to play a role in the dissemination of such novel variants. In this context, it is important to note that there is an increasing global trade in shellfish, which may enhance such dissemination.

FOOD PRODUCTION, PROCESSING, DISTRIBUTION AND CONSUMPTION

Most countries have enacted sanitary controls on the production of live bivalve molluscan shellfish. In the EU, these are covered by Council Directive 91/492/EEC (32) and in the United States, by interstate trading agreements set out in the FDA National Shellfish Sanitation Program Manual of Operation (33). The legislation requires that third country imports into EU and US have to be produced to the same standard as domestic products.

A major feature of these controls is the use of traditional indicators of faecal contamination, such as faecal coliforms or *E. coli*, either measured in the shellfish themselves (EU approach) or in the shellfish growing waters (US FDA approach). The microbiological standard of that less than 230 *E. coli* or 300 faecal coliforms in 100 g of shellfish flesh is internationally accepted and is based on a 5-tube 3-dilution most probable number (MPN test) which should be validated for shellfish matrix. It should be noted that viral standards are not currently set in EU or US legislation and that there is little correlation between coliform counts and viral contamination levels. Council Directive 91/492/EEC refers directly to the problem of viral contamination in shellfish and the need to introduce standards when such techniques become available (9). Since then, a network of reference laboratories for virus detection in shellfish has been established, but at present methods are not standardised yet.

Shellfish harvesting areas have been classified based on microbiological monitoring outcomes from clean areas (EU 'category A' and US FDA 'approved'), to contaminated areas (EU 'category B'; US FDA 'restricted') to heavily contaminated areas (EU 'category C') (9). Shellfish from clean areas can be taken for direct human consumption without further processing. Shellfish from contaminated areas ('B class') may only be placed on the market following commercial purification (purification) or relaying (transfer to cleaner water for self-purification), or after treatment by heat processing using an approved method. Shellfish from 'heavily contaminated areas ('C class') may only be placed on the market following protracted relaying or following commercial heat treatment by an approved method.

Several commercial heat treatment processes have been officially approved including the UK heat cook parameters of raising the internal temperature of shellfish meats to 90 °C for 1.5 min (Anon, 1993a)(10). This method seems to be effective for inactivating norovirus, but since norovirus cannot be cultivated, was only tested for hepatitis A virus and feline calicivirus, a possible model virus for norovirus. The degree of cooking required to reliably inactivate noroviruses would however probably render oysters unpalatable to consumers. The inability of home or restaurant cooking to provide adequate guarantees of consumer protection against viral contamination for bivalve shellfish emphasizes the reliance on harvesting and, for category B areas, depuration (see below)(9).

Depuration periods may vary from 1 to 7 days, however around 2 days is probably the widely used method (Lees, 2000) (7). Council Directive 91/492/EEC details requirements for approval of shellfish purification centers (9). Compliance with *E. coli* (or faecal coliform) end-product standards does however not provide a guarantee of virus absence as was demonstrated with documented outbreaks associated with depurated shellfish (7, 9). Viruses are eliminated from bivalve molluscs at a slower rate than faecal coliforms or *E. coli*. Viral removal during depuration seems to be dependent on different parameters, including the critically important temperature of the seawater that affects the activity of shellfish (9). Shellfish derived from restricted areas may be placed on the market once they comply with the microbiological standard for shellfish. These shellfish may however still be contaminated with pathogenic viruses, like norovirus, but also other enteric pathogens which may be even more difficult to relate with illness due to shellfish consumption.

The most effective way to tackle shellfish transmitted viral disease is to prevent or reduce sewage pollution of shellfish harvesting areas. Infrequent sanitary control monitoring programs provide however little protection against intermittent spills associated heavy rainfalls or fecal contamination related with water-recreation in production areas.

Limited information is available on consumer behaviour and the way these shellfish are prepared prior to consumption. However, Eurostat gives production and trade information figures, which give an impression of the total consumption in the EU of food products of particular interest. In 1998-1999, the bivalve molluscs production in EU given in tonnes live weight, including the weight of the shells, was about 35000 tonnes for wild caught shellfish and about 86000 tonnes for farmed shellfish. Data on the intra and extra EU imports as well as extra EU exports for 1998-2000, show that international trade is global and substantial (9).

RISK ASSESSMENT NEEDS AND QUESTIONS

The key needs in relation to the risk posed by the presence of norovirus in bivalve molluscan shellfish are as follows:

- for risk assessment purposes, information is needed on consumer behaviour and food preparation prior to consumption;
- for risk assessment purposes there is also a need for quantitative data on the presence of noroviruses in shellfish;
- information is needed on factors that can inactivate (noro)viruses in shellfish;
- there is a need for a cell culture system that will allow propagation of norovirus;
- given the low infectious dose of norovirus and the possible presence of other pathogenic viruses in shellfish especially from restricted areas, the question arises whether the practice for placing these shellfish on the market after depuration or relaying should be reconsidered;
- there is a need for improved surveillance of (foodborne) viral illness;
- there is a need for harmonized detection and genotyping to allow linking of patients and contaminated products.

AVAILABLE INFORMATION AND MAJOR KNOWLEDGE GAPS

PCR based procedures for the detection of noroviruses in shellfish are technically complex and currently not ready for routine food control laboratories. No standard internationally accepted methods (such as ISO) exist for shellfish extraction or norovirus RT-PCR, although the CEN/TC275/WG6/TAG4 (Microbiology of food and animal feeding stuffs, horizontal method for detection of norovirus and hepatitis A virus in food by RT-PCR) is preparing such protocols. Measuring the presence of RNA molecules by RT-PCR does not provide evidence that positively tested shellfish contain infectious particles. Here a drawback is that there are at present no *in vitro* culture systems for norovirus. On the other hand RNA molecules outside the viral capsid will be very instable. Moreover, positively tested shellfish samples can contain infectious particles as was demonstrated in outbreak studies with norovirus strains detected in the shellfish perfectly matching those detected in the stools of the patients (27-29). Only a few particles will be needed to produce illness.

CONCLUSIONS

Norovirus is an emerging infection that has been clearly linked with the consumption of raw or lightly cooked bivalve molluscan shellfish. Although the illness generally is considered mild and self-limiting, there is evidence that severe clinical features can occur in patients with several underlying diseases. Except for the secondary or tertiary waves of transmission that can be started after consumption of contaminated shellfish, consumption of shellfish containing a cocktail of viruses may even lead to the generation of recombinant norovirus strains which may have far-reaching epidemiological implications. After contact with fecally contaminated water the shellfish may also harbor other pathogenic viruses for which norovirus may act as an indicator. The permission of releasing shellfish derived from restricted

areas once they comply with microbiological standards, cause for concern as mentioned in this risk profile on the norovirus-shellfish commodity. Risk management strategies have to be developed in order to address the presence of viral contamination of these shellfish.

RECOMMENDED RISK MANAGEMENT ACTIONS

Considering the current state of knowledge related to this emerging foodborne pathogen, it is recommended that the Codex Committee on Food Hygiene undertake the following management activities.

Reassess the depuration procedure of shellfish from B and C-areas.

Stress the importance of implementing sanitary rules for waste disposal from ships or recreational sailing with all kinds of vessels in the neighbourhood of commercial shellfish areas and install appropriate discharge locations.

Mandatory reporting of viral food-related outbreaks through RASFF alerts.

Develop guidelines on the minimum level of evidence required to act upon suspected viral contamination in the absence of quantitative data on virus detection and viability in the implemented products.

Consequently, mandatory follow-up of viral food-related outbreaks through RASFF alerts.

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