A study on the occurrence of human enteric viruses in salad vegetables and seafood and associated health risks for consumers in Mauritius

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Abstract

Norovirus (NOV) and hepatitis A virus (HAV) are human enteric viruses of major concern worldwide. Salad vegetables and molluscan shellfish are highly susceptible to contamination by NOV and HAV and can pose a health threat when consumed raw. The objective of this study was to determine the occurrence of NOV and HAV in lettuce, watercress, tomatoes, and oysters using the enzyme-linked immunosorbent assay and assess the health risks associated with the consumption of these commodities by semi-quantitative risk assessment. The occurrence of NOV in vegetables ranked in the following decreasing order: lettuce (36%) > watercress (16%) > tomatoes (4%). However, HAV was more frequently detected in watercress (56%), compared to lettuce or tomatoes (12%). Additionally, NOV was detected in oysters (60%). The risk assessment exercise pointed to a medium-risk score of contracting a foodborne illness of viral origin for consumers eating fresh watercress or oysters. Future research will ascertain the presence of these enteric viruses in a broader range of food commodities.

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Introduction

Foodborne viruses have become a matter of concern for the food industry, and they are recognized among the top food safety priorities by risk assessment experts (Rowe and Bolger, 2016). Of the different viral agents responsible for foodborne illnesses, norovirus (NOV) and hepatitis A virus (HAV) are at present the most incriminated in foodborne illness cases and outbreaks. According to several sources, NOV is the most common cause of foodborne illness in the European region, with close to 15 million cases each year and more than 400 deaths (Ahmed *et al*., 2014; Cannon *et al*., 2021). A detailed analysis of gastroenteritis outbreaks in the US, conducted by the Centers for Disease Control and Prevention between 2009 and 2012, revealed that 48% of the outbreaks were due to NOV (Hall *et al*., 2014). It is interesting to note that 75% of these outbreaks resulted from food being consumed raw, and the items included leafy greens (30%), fresh fruit (21%) and shellfish (19%) (Hall *et al*., 2014). As far as HAV is concerned, the kinds of food that have been implicated in foodborne illness include oysters and raw fruits and vegetables. One potential way that fresh produce may become contaminated with HAV is through the use of water that is contaminated with fecal matter or the reuse of wastewater to irrigate crops (Stine *et al*., 2005). It has also been reported that HAV can adsorb on surfaces of fresh produce once it contaminates the food item and can remain infectious for days (Stine *et al*., 2005). In 2007, the US Food and Drug Administration conducted a market survey of oysters in the US which revealed that 3.9% of the oysters tested positive for NOV, while 4.4% tested positive for HAV (DePaola *et al.*, 2010).

NOV illness includes stomach pain, vomiting, diarrhea, and sometimes fever, while HAV can cause gastrointestinal upset, nausea, and liver infection with symptoms ranging from mild to severe (WHO, 2023). These symptoms can be more severe in immunocompromised hosts and, in rare cases, even cause death (WHO, 2023). Accurate and timely reporting of NOV and HAV infections is thus important due to the potential severity of the illness. In fact, disease manifestation varies with the etiological agent in question and the sensitivity of the infected people. Recently, risk assessment has become increasingly important as a means of providing an unbiased evaluation of the health risks associated with various food exposure scenarios. This is essential to prevent and control

hazards in food that may be susceptible to viral contamination. Both qualitative and quantitative assessments can be used to measure the likelihood and magnitude of exposure to such hazards. The illness severity can vary based on factors such as host immunity and susceptibility, variations in clinical symptoms and health outcomes, genetic diversity of the microorganism, and multiple potential routes of exposure (Bradshaw and Jaykus, 2016).

To our knowledge, no surveillance has been undertaken for foodborne viruses such as NOV and HAV in the food supply chain of Mauritius. Therefore, this study aimed to determine the occurrence of NOV and HAV in widely consumed fresh produce and shellfish commodities in Mauritius and assess the health risks posed by their consumption.

Materials and Methods

Sample collection

Samples comprised fresh produce [lettuce (*Lactuca sativa*) of the Mignonette variety, tomatoes (*Lycopersicon esculentum*) of the Romanella variety, and watercress (*Nasturtium officinale*)] and shellfish [oysters (*Saccostrea cuculata*)]. Tomatoes (n=25), lettuce (n=25), and watercress (n=25) were randomly sampled from various smallholder farmers as well as main marketplaces located in different regions of Mauritius, including Curepipe, Rose-Belle, St. Pierre, and Port-Louis, known to be highly frequented by customers. The sampling of lettuce took place during the summer season (October-February), while tomato and watercress were sampled during the winter season (August-September). Planters in Mauritius confirmed that the chosen seasons ensured the best availability of fresh produce items across the island. Oyster samples (n=10) were collected from an aquaculture farm located on the northern coast of Mauritius. Samples were aseptically collected in stomacher bags and transported immediately to the laboratory in a cooler bag for subsequent analysis by enzyme-linked immunosorbent assay (ELISA). Due to the high price of enzyme-linked immunosorbent assay (ELISA) kits and budgetary constraints, a larger number of samples could not be sampled and analyzed, as it would have been cost-prohibitive.

Enzyme-linked immunosorbent assay

The samples were pre-processed before testing. They were first rinsed with sterile water, and 10 g portions of each sample were homogenized and ground with phosphate-buffered saline extraction buffer (pH 7.4) in a clean mortar and pestle. The homogenates were collected in different labeled 15 mL corning tubes (Sigma Aldrich, St. Louis, MO, USA). Centrifugation of the mixture was then done for 20 minutes at 2000 RPM to separate the tissue debris. The supernatants obtained were collected and transferred to new corning tubes, which were then used for ELISA detection.

The ELISA kits of choice in this study were the human norovirus antigen (NOV-Ag) kit (Cat. No MBS167267, MyBioSource, San Diego, CA, USA) and the human HAV kit (Cat. No MBS3802415, MyBioSource, San Diego, CA, USA) for quick screening of NOV (genogroups 1 and 2) and HAV, respectively. These kits have been developed for research purposes for the rapid and qualitative detection of viral antigens from various matrices, including tissue homogenates (MyBioSource, San Diego, CA, USA). The mentioned kits were used following the manufacturer's instructions. The intra-assay/inter-assay precision of the NOV-Ag and HAV ELISA kits reported by the manufacturer was a coefficient of variation less than 12 and 15%, respectively. The quality control built into the assay included the use of a negative control and a positive control provided in the reagent kit. Unfortunately, it was not possible to use additional positive controls, in the form of food samples spiked with viral agents, besides the manufacturer's reagents test kit controls. This is because, to our knowledge, there are no laboratories in Mauritius undertaking cell culturing of NOV or HAV. Moreover, importing reference virus strains from an international laboratory would have been challenging, especially in the aftermath of the COVID-19 pandemic.

A microplate reader was subsequently used to read the optical density (OD) readings of the samples at 450 nm within 15 minutes of the completion of the last step of the assay. The validity of the assay was ensured by the average OD values of positive control wells being ≥1.00 and the average OD values of negative control wells being ≤0.15. The detection of the viral antigen in the samples was based on the critical (cut-off) value determined from the following equation (1):

$$\text{Critical (cut-off) value} = (\text{average of negative control wells}) + 0.15 \quad (1)$$

Samples with OD values greater than the cut-off value were classified as positive for NOV or HAV antigen. Conversely, those with OD values falling below the threshold were evaluated as negative. All samples were analyzed in triplicates.

Consumer survey and risk assessment

For commodities with a NOV or HAV detection rate exceeding 50%, a risk assessment was deemed important to estimate the risk of viral gastroenteritis associated with the consumption of these food items. A survey was first administered to 50 adult consumers to shed light on their patterns of consumption of the selected commodities (*Supplementary Survey Questionnaires 1 and 2*). The survey data collected was used to carry out a semi-quantitative microbial risk assessment using the spreadsheet-based tool Risk Ranger (Ross and Sumner, 2002).

This food safety risk calculation tool considers factors that can affect the magnitude of the risk caused by the hazard in a food commodity and converts qualitative descriptions into semi-quantitative variables by expressing them in mathematical terms (Duvenage and Korsten, 2017). The rate of NOV and HAV detection in these commodities as determined by ELISA was used to estimate the probability of product contamination (question 6 of the risk ranger). The variables in the Risk Ranger spreadsheet and the options used for the semi-quantitative risk assessment are shown in Tables 1 and 2. Risk was characterized as low when the Risk Ranger ranking (RRR) was less than 32, medium when the RRR was between 32 and 48, high when the RRR was between 48 and 60, and very high if the RRR exceeded 60 (Duvenage and Korsten, 2017).

Results and Discussion

In this study, various food items thought to be susceptible to contamination by NOV and HAV were screened for these agents using commercially available ELISA kits. Various authors (Atmar *et al*., 2018; Rabenau *et al*., 2003; Burton-MacLeod *et al*., 2004; Dimitriadis and Marshall, 2005) had previously made use of similar commercial ELISA kits for the detection of NOV or other viruses. De Bruin *et al*. (2006) mentioned that ELISA kits can actually

be useful for a preliminary screening of samples for viral contamination because of their rapidity and simplicity. Moreover, these enzyme immunoassays have been reported to have a high throughput and specificity (Zaczek-Moczydlowska *et al*., 2021) and a moderate overall analytical sensitivity (Kirby and Iturriza-Gómara, 2012). Indeed, this characteristic of the test really suits the purpose of this study since an estimation of the contamination rate was needed.

The occurrence of NOV in vegetables was ranked in the following decreasing order: lettuce (36%) > watercress (16%) > tomatoes (4%). The higher occurrence of NOV in lettuce could be due to its proximity to the soil medium during cultivation. Indeed, Brandl and Amundson (2008) have shown that foliar plants that grow in close contact with soil have a greater tendency to harbor enteric pathogens, which can multiply on the leaves of leafy greens such as lettuce crops. Additionally, a lower prevalence of pathogens was observed on aerial plant parts such as tomatoes. Nevertheless, the potential for contamination of aerial vegetables *via* splash dispersal of contaminated water or soil particles or *via* transmission by insect vectors cannot be ignored (Li *et al*., 2015). HAV was more frequently detected on watercress (56%) compared to lettuce or tomatoes (12%). Watercress is an aquatic plant that grows along the margins of slow-moving rivers, streams, ditches, and drains. Indeed, HAV infection has been epidemiologically linked to drinking unclean water or eating food that has been washed or grown in unclean water (Nasser, 1994). In Mauritius, watercress is grown commercially in waterways all over the island. They are usually cultivated in springs or rivers, which are often bordered by residential areas, and hence are susceptible to contamination by pathogens from human waste and animal feces, as hypothesized by Googoolee *et al*. (2020).

As far as oysters are concerned, NOV was present in 60% of samples tested, while HAV was undetectable. Bivalve shellfish, such as oysters, are vulnerable to viral contamination from the waters they are grown in. This poses a risk to human health as oysters are routinely consumed raw and untreated, making them a potential source of

Table 1. Information used for risk estimation of hepatitis A virus illness associated with raw and cooked watercress consumption.

Risk Ranger question	Details - raw watercress consumption	Details - cooked watercress consumption
1. Hazard Severity	Mild hazard - sometimes requires medical intervention	Mild hazard - sometimes requires medical intervention
2. Population susceptibility	General - all members of population	General - all members of population
3. Frequency of consumption	A few times per year	A few times per year
4. Portion of population consuming the product	Most (75%)	Most (75%)
5. Size of consuming population	975,000	975,000
6. Probability of contamination of raw product per serving	Common (50%)	Common (50%)
7. Effect of processing	The process has NO effect on the hazards	The process usually (99% of cases) eliminates the hazards
8. Potential for recontamination	No	No
9. Effectiveness of post-processing control system	NOT RELEVANT - level of risk agent does not change	NOT RELEVANT - level of risk agent does not change
10. Increase in post-processing contamination level to cause infection	Other (500-fold increase)	Other (500-fold increase)
11. Effect of food preparation before eating	Meal preparation has NO effect on the hazards	Meal preparation has NO effect on the hazards

Table 2. Information used for risk estimation of norovirus illness associated with fresh and cooked oyster consumption.

Risk Ranger question	Details - fresh oyster consumption	Details - cooked oyster consumption
1. Hazard severity	Mild hazard - sometimes requires medical intervention	Mild hazard - sometimes requires medical intervention
2. Population susceptibility	General - all members of population	General - all members of population
3. Frequency of consumption	A few times per year	A few times per year
4. Portion of population consuming the product	Some (25%)	Some (25%)
5. Size of consuming population	325,000	325,000
6. Probability of contamination of raw product per serving	Common (50%)	Common (50%)
7. Effect of processing	The process has NO effect on the hazards	The process usually (99% of cases) eliminates the hazards
8. Potential for recontamination	No	No
9. Effectiveness of post-processing control system	NOT RELEVANT - level of risk agent does not change	NOT RELEVANT - level of risk agent does not change
10. Increase in post-processing contamination level to cause infection	Slight (18-fold increase)	Slight (18-fold increase)
11. Effect of food preparation before eating	Meal preparation has NO effect on the hazards	Meal preparation has NO effect on the hazards

disease transmission (Battistini *et al*., 2021). Numerous outbreaks of viral illness in many countries have been linked to oysters. Additionally, Thebault *et al*. (2023) have reported that even oysters with low viral loads could be responsible for such outbreaks. The absence of HAV in the oyster samples tested suggests that HAV contamination may not be a real concern for shellfish farms in Mauritius.

To assess the health risks associated with the consumption of commodities having a high contamination rate (exceeding 50%) with foodborne viruses, consumer behavior information had to be gathered. Of the 50 survey participants recruited, 51% and 49% were of the male and female genders, respectively. Overall, 23% fell in the age bracket of 18-20, 36% between 21-30 years, 24% between 31-40 years, 10% between 41-50, and 7% fell in the range of 51-60 years of age. When asked about their oyster consumption patterns, 30% of the respondents indicated never consuming oysters, while 70% stated that they did consume oysters, albeit rarely. The supermarket or hypermarket was the most popular place for buying shellfish among respondents (34%), followed by markets (31%), seafood "cold storage" outlets (18%), and fish landing stations ("*debarcadere*") (17%). Of the shellfish-consuming participants, 41% mentioned consuming an oyster in one sitting, while 17% and 42% of respondents indicated a serving size of 2 units or more than 2 units during a meal, respectively. Approximately a third of the participants (34%) indicated consuming the bivalves fresh on the half-shell. On the other hand, 66% of the survey participants preferred to consume cooked oysters, with the most popular mode of cooking being shallow frying and steaming. Family or friends (83%) represented the most common induction link to consumption of the products, with the only other means being restaurants. None of the oyster-consuming participants indicated having any underlying medical conditions.

Data garnered from the survey on shellfish consumption patterns, such as serving size, frequency of consumption, method of preparation, *etc*., were then used for risk estimation by the risk assessment tool, Risk Ranger (Ross and Sumner, 2002). Given a 60% contamination rate of oysters by NOV, the semi-quantitative risk assessment exercise indicated that consumers eating raw shellfish earned a medium-risk ranking score of 37 for contracting a NOV infection. On the other hand, the risk was reduced to a low-risk score of 26 when oysters were subjected to a heat-killing step before consumption (Table 3). This risk score was estimated based on the frequency of exposure of the hosts (general members of the population) to an infectious dose of the agent and the severity of its infection. The probability of exposure to an infectious dose depends on: i) the serving size; ii) the probability of contamination of the raw product; iii) the initial level of contamination; iv) the probability of contamination at subsequent stages in the farm-to-fork chain; and v) changes in the level of hazard (viral load) during the journey from sea to plate (Ross and Sumner, 2002). The severity of infection, in turn, depends on several factors, including i) the frequency of contamination of shellfish by NOV; ii) the effect of processing and post-process control; iii) the proportion of people consuming the product raw; iv) their serving size and frequency of consumption; and v) the immunocompetency of the consumers. According to Duvenage and Korsten (2017), a score of less than 32 is considered a "low" risk, while values falling in the range of 32-48, 48-60 and >60 are deemed "moderate", "high" or "very high" risk scores, respectively. Sumner and Ross (2002) also similarly reported a comparable risk score of 31 for oysters contaminated with viruses. Based on the population size of Mauritius (*circa* 1.2 million) and the proportion of consumers eating raw oysters, this translates to *circa* 5 predicted illness cases of NOV-associated gastroenteritis per annum in the general population. Interestingly, when participants were queried about any past history of health-related issues following consumption of shellfish, 17% mentioned having suffered from food poisoning, although this could be attributed to other seafood hazardous agents. Taken together, the findings of the risk assessment exercise point to a moderate risk of viral foodborne illness associated with the consumption of raw oysters. Risk-mitigating measures that can be taken to further lower health risks include monitoring the environmental conditions of shellfish farms and the application of intervention strategies that include depuration, mild thermal treatment, thermal shock, as well as non-thermal processing technologies such as high-pressure processing (Lou *et al*., 2011).

As far as watercress is concerned, 20% of consumers indicated buying it from supermarkets or hypermarkets, 5% from vegetable hawkers or planters, while the majority (75%) purchased it from markets. The most important purchasing consideration for watercress was the absence of pests, such as snails, and off-odors of chemical products, such as herbicides. Moreover, the participants mentioned consuming watercress either raw, following minimal processing such as rinsing or soaking in water, or cooked. Li *et al*. (2015) mentioned that in Mauritius, watercress is traditionally served cooked, although it is increasingly being consumed raw in salads, sandwiches, or as a garnish. Minimal processing of watercress often does not involve steps that inactivate viruses, thereby heightening the infection risks (Bouwknegt *et al*., 2015). It is thus imperative to assess the risk of foodborne illness associated with the consumption of raw watercress contaminated by HAV. Based on a HAV occurrence rate of 56%, the risk assessment exercise pointed to a medium-risk score (40) for consumers eating fresh watercress without any heat-killing steps, with an estimated 8 illness cases per year. On the other hand, a low-risk ranking score was obtained when watercress was consumed following cooking. Of the 50 survey participants, none indicated having any underlying illnesses, but 16% of the respondents (8) mentioned having a previous history of health-related issues following consumption of watercress. However, this could have been largely underestimated since raw watercress is often included in dishes, such as salads and sandwiches, as a garnish rather than the main ingredient and could easily be overlooked during a dietary recall.

Table 3. Risk estimation.

Hazard	Food	Processing	Probability of illness per day per consumer of interest (Pinf×Pexp)	Total predicted illnesses/annum in population of interest	Risk ranking score	Ranking
NOV	Oyster	No cooking scenario	4.11E-08	4.88E+00	37	Medium
		Cooking scenario	4.11E-10	1.14E-02	26	Low
HAV	Watercress	No cooking scenario	4.11E-08	7.88E+00	40	Medium
		Cooking scenario	4.11E-10	7.88E-02	29	Low

NOV, norovirus; HAV, hepatitis A virus.

Conclusions

Taken together, it can be inferred that leafy salad vegetables such as lettuce and watercress, as well as molluscan shellfish, are common vehicles of enteric viruses. The relatively high occurrence of HAV and NOV in watercress and oysters, respectively, imply that appropriate cleaning and sanitation regimes must be adopted to avoid the introduction or persistence of these agents in the production systems. This is particularly important given that oysters and leafy greens are considered a delicacy for the Mauritian market and are generally consumed raw, posing a greater risk of contamination. The continued surveillance of foodborne viruses in these high-risk food matrices as well as other agricultural produce is greatly warranted. Given the heterogeneous distribution of viral particles in foods and the fact that they are present in smaller concentrations, more sensitive detection techniques should be used in the future to more accurately estimate the risk of infection from fresh produce and shellfish consumption.

References

Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, Koopmans M, Lopman BA, 2014. Global prevalence of norovirus in cases of gastroenteritis: a systematic review and meta-analysis. Lancet Infect Dis 14:725-30.

Atmar RL, Ramani S, Estes MK, 2018. Human noroviruses: recent advances in a 50-year history. Curr Opin Infect Dis 31:422-32.

Battistini R, Masotti C, Listorti V, Suffredini E, Maurella C, Garcia-Vozmediano A, Costa E, Lacona F, Orlandi M, Ercolini C, Serracca L, 2021. Norovirus persistence in oysters to prolonged commercial purification. Pathogens 10:944.

Bouwknegt M, Verhaelen K, Rzeżutka A, Kozyra I, Maunula L, von Bonsdorff CH, Vantarakis A, Kokkinos P, Petrovic T, Lazic S, Pavlik I, Vasickova P, Willems KA, Havelaar AH, Rutjes SA, de Roda Husman AM, 2015. Quantitative farm-to-fork risk assessment model for norovirus and hepatitis A virus in European leafy green vegetable and berry fruit supply chains. Int J Food Microbiol 198:50-8.

Bradshaw E, Jaykus LA, 2016. Risk assessment for foodborne viruses. In: Goyal SM, Cannon JL, eds. Viruses in foods. Springer, Cham, Switzerland, pp 471-503.

Brandl M, Amundson R, 2008. Leaf age as a risk factor in contamination of lettuce with Escherichia coli O157:H7 and Salmonella enterica. Appl Environ Microbiol 74:2298-306.

Burton-MacLeod JA, Kane EM, Beard RS, Hadley LA, Glass RI, Ando T, 2004. Evaluation and comparison of two commercial enzyme-linked immunosorbent assay kits for detection of antigenically diverse human noroviruses in stool samples. J Clin Microbiol 42:2587-95.

Cannon JL, Bonifacio J, Bucardo F, Buesa J, Bruggink L, Chan MCW, Fumian TM, Giri S, Gonzalez MD, Hewitt J, Lin J-H, Mans J, Muñoz C, Pan C-Y, Pang X-L, Pietsch C, Rahman M, Sakon N, Selvarangan R, Browne H, Barclay L, Vinje J, 2021. Global trends in norovirus genotype distribution among children with acute gastroenteritis. Emerg Infect Dis 27:1438-45.

De Bruin E, Duizer E, Vennema H, Koopmans MPG, 2006. Diagnosis of norovirus outbreaks by commercial ELISA or RT-PCR. J Virol Methods 137:259-64.

DePaola A, Jones JL, Woods J, Burkhardt W, Calci KR, Krantz JA, Bowers JC, Kasturi K, Byars RH, Jacobs E, Williams-Hill D, Nabe K, 2010. Bacterial and viral pathogens in live oysters: 2007 United States market survey. Appl Environ Microbiol 76:2754-68.

Dimitriadis A, Marshall JA, 2005. Evaluation of a commercial enzyme immunoassay for detection of norovirus in outbreak specimens. Eur J Clin Microbiol Infect Dis 24:615-8.

Duvenage S, Korsten L, 2017. Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer. Food Cont 78:374-82.

Googoolee AM, Takooree ST, Goburdhun D, Neetoo H, 2020. Characterizing the cultivation practices and microbiological quality of watercress. J Agric Food Res 2:100057.

Hall A, Wikswo M, Pringle K, Gould L, Parashar U, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, CDC, 2014. Vital signs: foodborne norovirus outbreaks - United States, 2009-2012. MMWR Morb Mortal Wkly Rep 63:491-5.

Kirby A, Iturriza-Gómara M, 2012. Norovirus diagnostics: options, applications and interpretations. Expert Rev Anti Infect Ther 10:423-33.

Li D, Keuckelaere AD, Uyttendaele M, 2015. Fate of foodborne viruses in the "farm to fork" chain of fresh produce. Compr Rev Food Sci Food Saf 14:755-70.

Lou F, Neetoo H, Chen H, Li J, 2021. Inactivation of a human norovirus surrogate by high-pressure processing: effectiveness, mechanism, and potential application in the fresh produce industry. Appl Environ Microbiol 77:1862-71.

Nasser AM, 1994. Prevalence and fate of hepatitis a virus in water. Crit Rev Env Sci Tec 24:281-323.

Rabenau HF, Stürmer M, Buxbaum S, Walczok A, Preiser W, Doerr HW, 2003. Laboratory diagnosis of norovirus: which method is the best? Intervirology 46:232-8.

Ross T, Sumner J, 2002. A simple, spreadsheet-based, food safety risk assessment tool. Int J Food Microbiol 77:39-53.

Rowe G, Bolger F, 2016. Final report on 'the identification of food safety priorities using the Delphi technique'. EFSA Supporting Publications 13:1007E.

Stine S, Song I, Choi CY, Gerba CP, 2005. Application of microbial risk assessment to the development of standards for enteric pathogens in water used to irrigate fresh produce. J Food Prot 68:913-8.

Sumner J, Ross T, 2002. A semi-quantitative seafood safety risk assessment. Int J Food Microbiol 77:55-9.

Thebault A, Teunis PFM, Le Pendu J, Le Guyader FS, Denis JB, 2013. Infectivity of GI and GII noroviruses established from oyster related outbreaks. Epidemics 5:98-110.

WHO, 2023. Hepatitis A. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-a.

Zaczek-Moczydlowska MA, Beizaei A, Dillon M, Campbell K, 2021. Current state-of-the-art diagnostics for norovirus detection: model approaches for point-of-care analysis. Trends Food Sci 114:684-95.

Online supplementary material:
Survey Questionnaire 1. Knowledge, perception and practices related to molluscan shellfish consumption.
Survey Questionnaire 2. General consumption preferences and practices in relation to the watercress vegetable.