Introduction

The Sardinian shellfish farming is a relevant and traditional economical aspect within the local economy. Most of production is mainly carried out in marine coastal areas with typical and geomorphic features, involving numerous human and manufacturing activities. Therefore, this marine environment becomes a potential source of seawater’s pollution and contamination for shellfish production. Bivalve molluscs may be contaminated by human sewage and they can constitute the main vector in the transmission of enteric diseases favouring the occurrence of situations of human health risk (Vilarino et al., 2009; Maalouf et al., 2010). The percentage of enteric viruses outbreak associated with shellfish is approximately 20% in countries such as United States, but this percentage increases up to 80% in countries in which seafood consumption is greater like Eastern areas or wherever seafood is eaten raw (Lees, 2000).

Foodborne diseases linked to the consumption of bivalve molluscs are mainly caused by enteric viruses, especially from enteric human Calicivirus [Norovirus (NoVs)] and hepatitis A virus (HAV) (Davies et al., 2001; Corrain et al., 2007); indeed, epidemiological indications suggest that human enteric viruses are the most common pathogens transmitted by bivalve shellfish (Lees, 2000). These viruses have the capability to persist in shellfish for several days (as far as 130 days in sea water), even if when this animals are placed in clean seawater used for growing or farming or depuration cycles (Crocì and Suffredini, 2003). Norovirus genogroup I (NoVG I) and II (NoVG II) have been detected in bivalve molluscs shellfish especially in oyster samples harvested and marketed from farm-pond and bays worldwide (Beuret et al., 2003; Cheng et al., 2005; Gallimore et al., 2005). In respect to shellfish market is regulated by European Union (EU) legislations (EC Regulations No. 852/04, No. 853/04, No. 854/04, No. 2073/05, No. 2074/05, No. 1881/06 and No. 1441/07; European Commission, 2004a, 2004b, 2004c, 2005a, 2005b, 2006, 2007). Specifically, Regulation of the European Commission (EC) No. 2073/05 (2005a) lays down the microbiological criteria based only on the determination of some bacteriological parameters (Salmonella spp. and Escherichia coli). However, the same regulation makes it clear that determining of faecal indicators is unreliable for demonstrating the presence or absence of viral contamination.

Shellfish from category A classification sites can be taken for direct human consumption. Shellfish from these sites must contain <230 E. coli bacteria per 100 g of flesh. Shellfish from category B classification (<4600 E. coli bacteria per 100 g of flesh for 90% of samples and <46,000 E. coli bacteria per 100 g of flesh for all samples) must be purified by relying or depuration prior to consumption. Shellfish from category C classification sites (all samples with 46,000 E. coli bacteria per 100 g of flesh) must be subjected to protracted relying (2 months) or commercial heat treatment prior to consumption (European Commission, 2004c).

The present work was carried out in accordance with the Regional Plan of Surveillance, Vigilance and Sanitary Control of Production and Marketing of Molluscs, and of Periodic Monitoring of Shellfish Farming Areas, in force in Sardinia region, during the period...
2008-2011 (Regione Sardegna, 2008). The aims of this work were i) to detect faecal indicators (*E. coli*) and NoVG, NoVGI, HAV contamination in shellfish samples collected from harvesting, growing, relaying areas in five production locations in Sardinia region, and ii) to evaluate a correlation between the presence of these contaminants and seasonality. Physicochemical parameters of all shellfish harvesting and relaying areas were measured. The data analysis obtained in this study is highly valuable for improving microbiological and viral contamination control of shellfish, increasing the level of understanding and safety for consumers, and helping aquaculture farming.

**Materials and Methods**

**Site selection and shellfish collection**

For this study to be representative of the regional country, samples from 1266 bivalve molluscs consisting of mussels (*Mytilus galloprovincialis*), oysters (*Crassostrea gigas*, *Ostrea edulis*), clams (*Tapes decussatus*, *Tapes philippinarum* and *Ruditapes philippinarum*, autochthonous native species in north Sardinian aquaculture) and *Cerastoderma* sp. or *Cardium* spp. were collected and tested for human enteric viruses and *E. coli*. Samples were collected in Sardinia region from five (Site 1 to 5) geographically separate harvesting, growing and relaying areas, classified from A (product suitable for direct consumption) to C (product suitable for consumption after long depuration treatment) (European Commission, 2004c). Each area was in marine coast and pond locations with considerable urban populations (>50,000) (Figure 1). Shellfish was harvested by local authority sampling officers in accordance with the official monitoring and surveillance programme and in compliance with the Shellfish Regional Plan (Regione Sardegna, 2008). Sampling officers collected shellfish per week/month from each selected site between January 2009 and December 2011 included. Each sample consisted of a variable number of individuals (20-30), based on size and species. Samples were shipped directly to laboratory via cold storage in 12 h and immediately tested for *E. coli*, while tested on different days for viral contamination (HAV, NoVs).

**Escherichia coli analysis**

*Escherichia coli* was isolated using most probable number (MPN) standard methods as described in ISO TS 16649-3:2005 (ISO, 2005b). Calculation of the number of *E. coli* was performed using the ISO 7251 MPN table (ISO 7251:2005; ISO, 2005a). Processed shellfish was stored at -80±1.0°C and later used for enteric virus detection by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) as described by Croci (2009) and at a later time by Lees and CEN WG6 TAG4 Working Grasp (2010). While workable, environmental parameters, in particular water temperatures, at the sampling site were determined by using a mobile equipment always approved by the competent authority at the time of sampling.

**Shellfish processing for virus detection and viral RNA extraction**

Shellfish samples were washed under clean running water and opened with sterile equipment. For each sample, the digestive glands and hepatopancreas of 10/20 shellfish were excised, pooled, and then blended by using T 25 basic Ultra-Turrax®. From the original homogenate 2.0±0.1 g of chopped glands were transferred into a clean tube, and inoculated with 10⁶ 50% tissue culture infective doses feline calicivirus (FCV) (10 µL). Feline calicivirus was chosen as the sample process control virus (Mattison et al., 2007; Di Pasquale et al., 2010). Homogenates were prepared by treating the glands with 100 g/mL proteinase K solution (30 U/mg; 2.0±0.1 mL for each sample) (Promega, Madison, WI, USA). The samples were then incubated at 37±1.0°C with shaking at 300 rpm for 1 h and then incubated at 60±1.0°C for 15±1 min in a water bath. Finally, the sample was centrifuged at 3000 g for 5 min, the soluble portion (homogenate) was retained for testing, and the pellet was discarded. Homogenates were stored at -80±1.0°C prior to testing. Total RNA was extracted from 140 µL of shellfish homogenate by using mini-kits QiAamp® Viral RNeasy (Qiagen, Valencia, CA, USA) following the manufacturer’s instructions (RNA was eluted in 60 µL and was stored at -80±1.0°C until testing).

**Detection of Norovirus genogroup I and II and hepatitis A virus by real-time TaqMan reverse transcriptase polymerase chain reaction**

For NoVG, primers QNIF4 (da Silva et al., 2007) and NV1LR (Svraka et al., 2007) and probe NVGG1 (Svraka et al., 2007) were used. For NoVGI primers, NOF2 (Loisy et al., 2005), COG2R (Kageyama et al., 2003) and probe QNIFS (Loisy et al., 2005) were used. The two probes for NoVG and NoVGI were labeled 5’-6-carboxyfluorescein and 3’-6-carboxytetramethylrhodamine. Hepatitis A virus assay was performed with primers HAV68, HAV240 and HAV151.
Table 1. Positive shellfish samples tested for microbiological parameters.

| Period (total no. of samples tested) | Site | Samples (no. of positive targets) | Classification | No. of contaminated samples (%) |
|--------------------------------------|------|----------------------------------|----------------|---------------------------------|
|                                      |      |                                  |                | E. coli  | HAV | NoVGII | NoVGII+NoVGII |
| 2009 (205)                           | 1    | Musel (92)                        | B              | 7 (8)   | 15 (16) | 5 (5) |
|                                      |      | Oyster (12)                       | A/relying      | 2 (17)  | 2 (17)  | 1 (8) |
|                                      | 3    | Mussel (35)                       | B              | 1 (3)   | 4 (11)  | 1 (3) |
|                                      |      | Clam (30)                         | A/B            | 1 (3)   | 1 (3)   | 1 (3) |
|                                      | 5    | Mussel (11)                       | B              | 6 (20)  | 1 (9)   |        |
| 2010 (473)                           | 1    | Mussel (175)                      | B              | 11 (6)  | 76 (43) | 5 (3) |
|                                      |      | Oyster (21)                       | A/B            | 1 (5)   | 4 (19)  | 1 (5) |
|                                      | 3    | Mussel (59)                       | A/B            | 4 (8)   |        |        |
|                                      |      | Clam (20)                         | A/B/C          | 2 (10)  |        |        |
|                                      | 5    | Mussel (181)                      | B              | 6 (3)   | 17 (9)  | 5 (3) |
| 2011 (588)                           | 1    | Mussel (161)                      | B              | 6 (4)   |        | 59 (37) |
|                                      |      | Oyster (20)                       | A/B            | 2 (10)  | 10 (50) |        |
|                                      | 2    | Mussel (40)                       | B              | 6 (15)  | 8 (29)  |        |
|                                      |      | Mussel (45)                       | A/B            | 1 (2)   | 5 (11)  |        |
|                                      | 3    | Clam (43)                         | A/B/C          | 15 (35) | 8 (17)  |        |
|                                      | 5    | Mussel (224)                      | B              | 11 (5)  | 62 (28) |        |
|                                      |      | Clam (40)                         | B              | 1 (3)   | 4 (10)  |        |

E. coli, Escherichia coli; HAV, hepatitis A virus; NoVGII, Norovirus genogroup I; NoVGIII, Norovirus genogroup II; A, product suitable for direct consumption; B, product suitable for consumption after depuration treatment; C, product suitable for consumption after long depuration treatment.

### Results

The results of environmental monitoring showed that the Sardinian seawater had a stable salinity in all areas investigated. Salinity fluctuated between 27.0±1.0 and 36.0±1.0‰ (average=32.26‰). The seawater temperature during all monitoring varied between 11.0±1.0°C in January and 26.0±1.0°C in August. Dissolved oxygen and pH parameters were substantially equivalent in the five areas under observation during the same monitoring time, showing no significant variation during all three years of analysis.

In this study, 112 samples were taken from sites with a category A classification at the time of sampling, 1139 were taken from sites with a category B classification, and 15 were taken from sites with a category C classification. From a comparison of average E. coli and average NoVs levels in samples collected from the same site showed 30% of a correlation in B areas. The presence of E. coli during the study was 3.8% (49 samples positive/1266 samples analysed) and it most frequently occurred (3.7%; 22 positive samples/588 samples...
analysed) in the spring season of 2011 (Tables 1 and 2). The studied A class areas showed levels of E. coli higher than 230 MPN/100 g FIL in 6 clam samples. In the studied B class areas 40 shellfish samples showed all values well above of 4600 MPN/100 g FIL with a mussel sample with 30,000 MPN/100 g FIL. Interesting 11 (1.8%) mussels sampled in Site 5 showed E. coli levels far above the European legislation limit (4600 MPN/100 g FIL) (data not shown). With respect to the water temperature a correlation analysis has been performed. At temperatures between 10.0 ± 1.0 and 20.0 ± 1.0°C E. coli presence did not show significant correlation with seawater temperatures during the three years of the study. On the contrary, in 2011 E. coli at temperatures <19.0 ± 1.0°C showed highest counts (Table 2). In respect to sampling shellfish monitoring plan 1266 samples were tested for human enteric viruses and all provided valid results (acceptable extraction and amplification efficiencies). Of these samples, 337 (26.6%) tested positive for NoVs, with 19 (1.5%) being positive for both genogroups, 29 (2.2%) being positive for NoVGI only, and 289 (22.8%) being positive for NoVGGI only. The NoVs prevalence showed a high seasonal variability, with a maximum level of positive samples between January and March in 2009, 2010 and 2011 and a minimum of 0.15% positive samples (2/1266) in August 2009 (Table 2; Figure 1). Prevalence of NoVs levels varied markedly by season, with annual peaks occurring between January and March and the lowest levels being recorded between July and August (Table 2). Levels of NoVGGI were on average higher than those of NoVGI. For each month of the study, the percentage of samples with total NoVs (GI plus GII summed) in different animal species is shown in Table 1 in which the impact of possible control limits on NoVs levels in production areas at different times of the year is remarkably shown. All five sites sampled during the research returned at least one positive result. Year-by-year NoVs prevalence varied between 20 and 27.9% positive samples in 2010. Site-by-site NoVs prevalence varied from a minimum of 1.2% (64/73 samples tested) up to 16.9% positive samples (80/473 samples tested) in 2010. We observed higher frequency of NoVs in B Class areas (Table 1). The results obtained in relation to a large number of analysis showed interesting analytical data, in fact, hepatitis A virus was found only in a mussel sample collected from the Site 5, during the winter season in 2009 (Tables 1 and 2). Among the NoVs positive samples 2 (0.9%) were collected from a relaying water production area in Site 1 (oysters) in 2009, 4 (0.8%) were collected from a class C water production areas in Site 1 (oysters) and 4 (0.8%) in Site 3 (mussels) in 2010. Only one clam sample was positive for NoVGGI from a class C water production area in Site 3 in 2010. Logistic regression showed that there is a risk to find NoVs positive samples, in Site 1, 3.37 times higher than the Site 5 (Site 5 is the reference by algorithm calculation, OR=1) and other areas have not achieved significant results.

Discussion and Conclusions

In Sardinia region, one of the most important producer in Italy, shellfish production is a significant economic activity, with producing areas distributed along different coasts. Marine areas evaluated in this study support active commercial and recreational shellfish growing and harvesting activities. Most of the shellfish produced are consumed in local trade, especially in the western Sardinian coastal area, and in the North continental Italy (Sardegna Agricoltura/Laore, 2009). Shellfish farmers sell their products directly from the farms to wholesale, national and regional market and retail. During the research we did not observe, like in other countries, human contamination by NoVs (in Sardinia region, at present, clinical cases have not been recorded). Our study evaluated the contamination throughout the three years of the study and observed the probable presence of NoVs levels in shellfish during winter and summer period comparing this data to the environmental parameters (Figure 1; Table 2). The results of our research have shown no correlation between E. coli concentration and NoVs presence. The result clearly showed the absence of correlation between bacterial and viral faecal contaminants. In 2010 and 2011 the frequencies of NoVs positive samples were 26 and 23% in mussels respectively, in winter season, as the data confirm, even though we detected both NoVs genogroups in shellfish that were collected even in summer. The occurrence of frequencies NoVs positive samples in shellfish lead to think of the importance of regional or local conditions, particularly faecal contamination sources. Levels of NoVs contamination were observed at all sampling locations (except in the Site 2 and Site 4 in 2009 and in 2010) in wintertime, especially in Site 2, but this is not a real seasonality. The measures of the environmental parameters in the five harvesting, growing and relaying areas showed regular fluctuations related to seasonal variations, in particular the temperature (Figure 1). Most of NoVs contamination from Site 1 to 5 were detected during the winter season and no samples resulted positive during 2010 for E. coli contamination. We observed the high coincidence of two genogroups (GI, GII) in mussels (10%) in Site 1 showing that, along with all the other data, this area was especially NoVs-sensitive. The risk of finding mussels positive for NoVs in the year 2011 compared to the year 2010 was significantly higher (Table 3), while there was no statistically significant difference in the type of samples (clams, mussels).
The analysis of the variable month confirms the above data with high risks, statistically significance, and a positive outcome between November and March, with a peak between January and February. Regarding the temperature, the greatest risk is evident in the range between 11.0±1.0 and 15.0±1.0°C but it was not statistically significant. The data analysis showed that the concentration of NoVs is greater in winter highlighting accordingly that the presence of this organism shows a certain seasonality (Tables 2 and 3), in agreement with a study conducted in 2002 in Northern Italy from human cases in 2001 and 2002, in agreement with a study conducted in 2002 in Northern Italy from human cases in 2002. Several reports in fact have been published describing outbreaks of acute gastroenteritis in humans related to the consumption of raw shellfish (oysters) (Svraka et al. 2007). One reason could be that the shellfish metabolism changes from winter to summer and in this season there is more animal faecal contamination in shellfish growing areas (Lowther et al., 2012).

The data obtained in this study could contribute to the introduction of explicit shellfish monitoring programmes, which could be improved with acquisition of all environmental parameters data and real-time qRT-PCR tests on cultured areas. Optimisation of monitoring plans could lead to better management of the shellfish harvest and market.

Table 3. Odds ratio values and their confidence intervals (95%) defined by logistic regression.

| Variable   | SE     | OR     | 95% CI, lower limit | 95% CI, upper limit |
|------------|--------|--------|---------------------|---------------------|
| Year 2009  | 0.29909| 0.447854| -0.13836            | 1.03407             |
| Year 2010  | 0.171556| 0.396681| 0.060823            | 0.732539            |
| Site 1     | 0.196746| 3.37382 | 2.988198            | 3.759442            |
| Site 2     | 0.356512| 0.963479| 0.264715            | 1.662243            |
| Site 3     | 0.270447| 0.393788| 0.497127            | 1.463864            |
| Site 4     | 123.389 | 1.1877E-05| -241.842           | 241.8425            |
| E. coli    | 0.193196| 0.322087| -0.05658            | 0.700751            |
| January    | 1.1615  | 294.58  | 292.303             | 296.8565            |
| February   | 1.16329 | 864.347 | 862.067             | 866.627             |
| March      | 1.14057 | 324.475 | 322.2395            | 326.7105            |
| April      | 1.08608 | 113.121 | 110.9923            | 115.2497            |
| May        | 1.03065 | 60.0302 | 58.0103             | 62.05027            |
| June       | 0.987417| 52.7993 | 50.8639             | 54.73464            |
| July       | 0.61474 | 1.11993 | -0.08496            | 2.32482             |
| August     | 0.728953| 0.554156| -0.87458            | 1.982916            |
| October    | 0.952403| 10.9379 | 9.07119             | 12.80461            |
| November   | 1.09492 | 53.1344 | 50.98806            | 55.28014            |
| December   | 1.11818 | 103.255 | 101.0634            | 105.4466            |
| 11.00-14.90°C | 1.3498 | 0.0097962| -2.63581            | 2.655404            |
| 15.00-18.90°C | 1.32932| 0.034204| -2.57124            | 2.639688            |
| 19.00-22.90°C | 1.26068| 0.022485| -2.44845            | 2.493418            |
| 23.00-26.90°C | 0.857982| 0.154801| -1.52684            | 1.835446            |
| Clam       | 197.9   | 0.0001793| -387.584            | 387.8841            |
| Mussel     | 0.267597| 0.622397| 0.097907            | 1.146877            |
| Oyster     | 0.437888| 0.313003| -0.54528            | 1.171233            |

SE, standard error; OR, odds ratio; CI, confidence interval.

References

Beuret C, Baumgartner A, Schuep J, 2003. Virus-contaminated oysters: a 3-month monitoring of oysters imported to Switzerland. Appl Environ Microb 69:2292-7.

Boxman ILA, Tilburg JJHC, Te Loeke NAJM, Vennema H, Jonker H, de Boer E, Koopmans M, 2006. Detection of noroviruses in shellfish in the Netherlands. Int J Food Microbiol 108:391-6.

Cheng PKC, Wong DKK, Chung TWH, Lim WWL, 2005. Norovirus contamination found in oysters worldwide. J Med Virol 76:593-7.

Corrain C, Anganelli G, Fasolato L, Manfrin A, Rossetti E, Piazzio E, Mioni R, Pavoni E, Losio N, Sanavio G, Suffredini E, Croci L, 2007. [Influenza climatico ambientali sulla presenza di virus enterici in molluschi bivalvi]. [Article in Italian]. Ind Aliment 46:277-83.

Costafreda MI, Bosch A, Pintó RM, 2006. Development, evaluation, and standardization of a real-time TaqMan reverse transcription-PCR assay for quantification of hepatitis A virus in clinical and shellfish samples. Appl Environ Microb 72:3846-55.

Croci L, 2009. Determinazione di Norovirus ed HAV in molluschi bivalvi mediante Real Time PCR. Available from: http://www.iss.it/binary/spva/cont/Determinazione_NoV_e_HAV_Integrazioni_Correzioni_Dichiaraizione_validazione_Tabella _comparativa_POMIAC01.002.pdf

Croci L, Suffredini E, 2003. [Rischio microbiologico associato al consumo di prodotti ittici]. Available from: http://www.iss.it/publ/anna/2003/1/39135.pdf

da Silva AK, Le Saux JC, Paranaudeau S, Pompeuy M, Elinechelb M, Le Guyader FS, 2007. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. Appl Environ Microb 73:7891-7.

Davies AR, Capell C, Jehanno D, Nychas GJE, 2006. Evaluation of foodborne pathogens on European fish. Food Control 12:67-71.

Di Pasquale S, Paniconi M, De Medici D, Suffredini E, Croci L, 2010. Duplex Real Time PCR for the detection of hepatitis A virus in shellfish using Feline Calicivirus as a process control. J Virol Methods 163:96-100.

European Commission, 2004a. [Regolamento del Parlamento Europeo e del Consiglio del 29 aprile 2004 sull’igiene dei prodotti alimentari, 852/2004/CE]. [Regulation in Italian]. In: Official Journal, L 139/1, 30/04/2004.

European Commission, 2004b. [Regolamento del Parlamento Europeo e del Consiglio del 29 aprile 2004 sull’igiene dei prodotti alimentari, 852/2004/CE]. [Regulation in Italian]. In: Official Journal, L 139/55, 30/04/2004.

European Commission, 2004c. Commission regulation of 29 April 2004 laying down specific rules for the organization of offi-
cials on products of animal origin intended for human consumption, 854/2004/EC. In: Official Journal, L 139, 25/06/2004.

European Commission, 2005a. [Regolamento della Commissione del 15 novembre 2005 sui criteri microbiologici applicabili ai prodotti alimentari, 2073/2005/CE]. [Regulation in Italian]. In: Official Journal, L 338/1, 22/12/2005.

European Commission, 2005b. [Regolamento della Commissione del 5 dicembre 2005 recante modalità di attuazione relative a taluni prodotti di cui al regolamento (CE) n. 853/2004 del Parlamento europeo e del Consiglio e all’organizzazione di controlli ufficiali a norma dei regolamenti del Parlamento europeo e del Consiglio (CE) n. 854/2004 e (CE) n. 882/2004, deroga al regolamento (CE) n. 852/2004 del Parlamento europeo e del Consiglio e modifica dei regolamenti (CE) n. 853/2004 e (CE) n. 854/2004, 2074/2005/CE]. [Regulation in Italian]. In: Official Journal, L 338/27, 22/12/2005.

European Commission, 2006. [Regolamento della Commissione del 19 dicembre 2006 che definisce i temi massimi di alcuni contaminanti nei prodotti alimentari, 1881/2006/CE]. [Regulation in Italian]. In: Official Journal, L 364/5, 20/12/2006.

European Commission, 2007. [Regolamento della Commissione del 5 dicembre 2007 che modifica il regolamento (CE) n. 2073/2005 sui criteri microbiologici applicabili ai prodotti alimentari, 1441/2007/CE]. [Regulation in Italian]. In: Official Journal, L 322/12, 07/12/2007.

Gallimore CI, Cheesbrough JS, Lamden K, Bingham C, Gray JJ, 2005. Multiple norovirus genotypes characterised from an oyster-associated outbreak of gastroenteritis. Int J Food Microbiol 103:323-30.

ISO, 2005a. Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of presumptive Escherichia coli. Most probable number technique. ISO Norm 7251:2005. International Standardization Organization ed., Geneva, Switzerland.

ISO, 2005b. 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. ISO Norm 16649:2005. International Standardization Organization ed., Geneva, Switzerland.

Kageyama T, Kojima S, Shinohara M, Uchida K, Fukushima S, Hoshino FB, Takeda N, Katayama K, 2003. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. J Clin Microbiol 41:1548-57.

Lees DN, 2000. Viruses and bivalve shellfish. Int J Food Microbiol 59:117-26.

Lees DN, CEN WG6 TAG4 Working Grasp, 2010. International standardization of a method for detection of human pathogenic viruses in molluscan shellfish. Food Environ Virol 2:146-55.

Loisy F, Atmar RL, Guillon P, Le Cann P, Pommpeny M, Le Guyader FS, 2005. Real time RT-PCR for norovirus screening in shellfish. J Virol Methods 123:1-7.

Lowther JA, Gustar NE, Powell AI, Hartnell RE, Lees DN, 2012. Two-year systematic study to assess Norovirus contamination in oysters from commercial harvesting areas in the United Kingdom. Appl Environ Microb 78:5812-7.

Maalouf H, Zakhour M, Le P endu J, Le Saux JC, Atmar RL, Le Guyader FS, 2010. Distribution in tissue and seasonal variation of Norovirus genogroup I and II ligands in oysters. Appl Environ Microb 76:5621-30.

Mattison K, Brassard J, Houde A, Simard C, Pagotto F, Jones T, Trottier Y, Octobre I, 2007. The feline calicivirus (FCV) as an internal control for the detection of RNA viruses from foods. Federal Food Safety and Nutrition Research Meeting Publ., Winnipeg, Manitoba, Canada.

Medici MC, Martinelli M, Abelli LA, Ruggeri FM, Di Bartolo I, Arcangeli MC, 2006. Molecular epidemiology of Norovirus infections in sporadic cases of viral gastroenteritis among children in northern Italy. J Med Virol 78:1486-92.

Regione Sardegna, 2008. [Piano regionale per la vigilanza ed il controllo sanitario della produzione e commercializzazione dei molluschi bivalvi vivi e per il monitoraggio periodico delle zone di produzione e di stabulazione di molluschi bivalvi vivi, anno 2008-2011]. [Regulation in Italian]. Regione Sardegna Publ., Cagliari, Italy.

Sardegna Agricoltura/Laore, 2009. [Il comparto dell’acquacoltura in Sardegna alla luce dei risultati dell’indagine conoscitiva]. [Article in Italian]. Available from: http://www.sardegnanaagricoltura.it/documenti/14_43_20091201175044.pdf

Svraka S, Duizer E, Vennema H, de Bruni E, van der Veer B, Dorresteijn B, Koopmans M, 2007. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. J Clin Microbiol 45:1389-94.

Vilariño ML, Le Guyader FS, Polo D, Schaeffer J, Kröl J, Roman del JL, 2009. Assessment of human enteric viruses in cultured and wild bivalve mussels. Int Microbiol 12:145-51.

Ward P, Poitrans E, Leblanc D, Letellier A, Brassard J, Plante D, Houde A, 2009. Comparative analysis of different TaqMan real-time RT-PCR assays for the detection of swine Hepatitis E virus and integration of Feline calicivirus as internal control. J Appl Microbiol 106:1360-9.