Monitoring Shellfish harvesting areas for Norwalk-Like Viruses (NLVs): Implications for combating the transmission of these viruses by molluscan shellfish

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Introduction

Increasing evidence suggests that NLVs are a significant cause of gastroenteritis in the adult population. Although person to person transmission probably accounts for the bulk of cases, spread by the faecal-oral route through food or water vectors is likely to be important in the further dissemination of these viruses into vulnerable populations. Preventing such index cases may prove significant in combating this major cause of adult gastroenteritis. Waterborne transmission of NLVs through faecal pollution of the aquatic environment is well documented through contamination of vectors such as molluscan shellfish. The shortage of pristine conditions dictates that in developed countries many marine water sources used for shellfisheries and recreational purposes are inevitably faecally contaminated. Legislative controls based on conventional bacterial indicators have failed to prevent shellfish transmitted outbreaks of viral-gastroenteritis caused by NLVs. Monitoring shellfish harvesting areas for NLV contamination may provide an alternative approach for more effective public health controls. We describe the application of a nested NLV RT-PCR for monitoring viral contamination in a polluted oyster harvesting area over a one year period. The harvesting area was classified as Category B under EC legislation however it was known to be faecally polluted and to have been implicated in outbreaks of viral gastroenteritis. All oysters harvested from this area were purified (depurated) before sale for consumption. RT-PCR positive amplicons were characterised by cloning and sequence analysis. The public health significance of findings on the prevalence and diversity of NLV strains contaminating this shellfishery is discussed in relation to the potential of this approach for more effective control of shellfish associated viral-gastroenteritis.

Materials and Methods

Harvesting area studies. Samples of 24 oysters were obtained from a commercial producer both before and after depuration on a weekly to fortnightly basis. All samples were received in the laboratory within 48 hours. On receipt 10 oysters were assayed for E.coli using standard methods (Dore and Lees, 1995) and the remaining oysters frozen at -20°C for subsequent analysis for NLVs.

Shellfish Processing, virus extraction and purification and extraction of viral RNA. These procedure have been previously described in full (Lees et al., 1994).

Nested NVL RT-PCR. This method and its application to shellfish samples has been fully described previously (Lees et al., 1995, Green et al., 1998). Essentially the nested PCR uses 3 primers in the first round amplification, and two Genogroup specific primer sets in the nested PCR. They are known to detect approximately 90% of the NLVs currently circulating in the UK.

Cloning and sequencing. All RT-PCR positive amplicons were separated from unincorporated oligonucleotide primers and nucleotides using Chromaspin 100 columns (Clontech Laboratories Inc) ligated into a pGem vector and transformed (pGem-T Vector System, Promega). Colonies from each sample were screened for inserts using colony PCR. A minimum of 5 positives clones from each sample were further purified using microsep 30k columns (Filtron Technology Corporation, MA), and both DNA strands sequenced using the ABI PRISM™ dye terminator cycle sequencing system (Perkin Elmer) and analysed on an ABI 310 genetic analyser.

Sequence analysis. Sequence data analysis was performed using the MegaAlign and EditSeq components of the Lasergene software (DNAStar, London U.K).

Results

Table 1. Summary of E. coli and NVL results.

BEFORE DEPURATION				AFTER DEPURATION			
Date	*E.Coli*	RT-PCR	Confirmed (by sequence analysis)	Date	*E.Coli*	RT-PCR	Confirmed (by sequence analysis)
08-Aug 95	2400	+	Ongoing	08-Aug 95	<20	-	
15-Aug 95	>18000	+	Ongoing	17-Aug 95	<20	-	
22-Aug 95	>18000	-		24-Aug 95	<20	-	
30-Aug 95	2400	-		01-Sep 96	<20	-	
13-Sep 95	3100	-		15-Sep 95	<20	-	
26-Sep 95	220	+	Yes	28-Sep 95	<20	-	
10-Oct 95	2400	-		13-Oct 95	<20	-	
24-Oct 95	5400	-		26-Oct 95	<20	-	
07-Nov 95	5400	-		09-Nov 95	<20	-	
21-Nov 95	1100	-		28-Nov 95	<20	-	
09-Jan 96	1100	+	Yes	12-Jan 96	<20	+	tbd
16-Jan 96	110	+	Ongoing	18-Jan 96	70	+	Yes
23-Jan 96	200	+	Yes	25-Jan 96	20	+	Yes
31-Jan 96	10	+	tbd	02-Feb 96	<20	+	Yes
06-Feb 96	500	+	Yes	08-Feb 96	<20	+	Yes
14-Feb 96	700	-		16-Feb 96	<20	-	
20-Feb 96	500	+	Yes	22-Feb 96	<20	+	Yes
28-Feb 96	110	+	Yes	01-Mar 96	<20	+	Yes
06-Mar 96	5400	+	Yes	08-Mar 96	150	+	Ongoing
12-Mar 96	750	+	Yes	14-Mar 96	90	-	
19-Mar 96	310	+	Yes	22-Mar 96	<20	+	Yes
27-Mar 96	<20	+	Yes	29-Mar 96	<20	+	Yes
16-Apr 96	1300	+	Yes	18-Apr 96	<20	-	
30-Apr 96	1300	-		02-May 96	<20	-	
05-Jun 96	<20	-		07-Jun 96	<20	-	
18-Jun 96	230	-		20-Jun 96	<20	-	
16-Jul 96	<20	-		19-Jul 96	<20	-	
13-Aug 96	22000	-		15-Aug 96	<20	-	
03-Sep 96	310	-		05-Sep 96	<20	-	
17-Sep 96	220	+	tbd	19-Sep 96	<20	-	
01-Oct 96	<20	+	tbd	04-Oct 96	<20	+	tbd
16-Oct 96	70	+	tbd	18-Oct 96	<20	+	tbd

tbd = to be done

Table 1 tabulates E.coli and NLV results for all samples tested in this survey. The inadequacy of E. coli as an indicator of the viral risk associated with depurated shellfish is clearly demonstrated in table 1. Whilst high levels of E. coli were present in the majority of oysters sampled before depuration all samples were subsequently found to contain E. coli levels below the end-product standard of <230/100g following depuration. By contrast NLVs were found to be present in oysters both before (56% positive) and after (38% positive) depuration.

Figure 1 shows NLV PCR results for selected matched pairs (before and after purification) of samples taken throughout the study period. These results are presented chronologically in figure 2 together with the known outbreaks of gastro-enteric food poisoning associated with oysters from this harvesting area during the study period.

A clear seasonal difference can be observed in numbers of NLV positive oysters with the largest numbers of positive samples (73%) obtained during the winter period (October to March inclusive). This data concurs with the known winter association of gastro-enteric illness due to oyster consumption in the UK and also with the majority of the outbreaks associated with shellfish harvested from this area during the study period (figure 2). It is also noticeable that, for the most part, outbreaks from this harvesting area correlated with prior detection of NLVs in monitored oysters. Of the NLV positive samples during the winter period virus was successfully eliminated from only two samples during depuration with 63% of samples remaining positive (table 1 and figure 1). This concurs with evidence from many outbreaks showing that although bacterial indicators can be removed by depuration human enteric viruses are more problematical. By contrast the prevalence of NLVs in oysters was considerably lower in the summer period (April to September inclusive) with only 31% of samples containing NLVs. Interestingly depuration also appeared to be more efficient during the summer with NLVs being successfully eliminated from all four NLV positive samples following depuration (figure 1 and table 1). This is a novel finding which has potential significance for further studies on virus elimination during depuration.

Figure 1. Gel electrophoresis of NLV RT-PCR amplicons from selected paired (before and after depuration) oyster samples throughout study period.

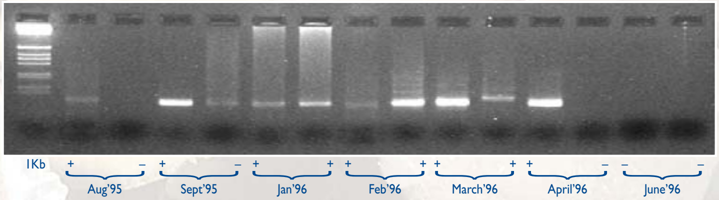
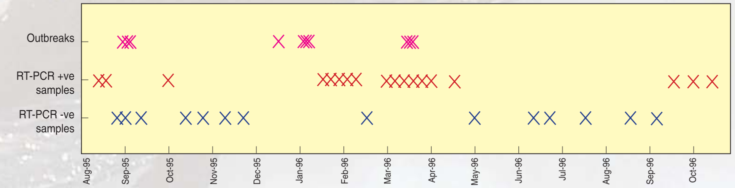


Figure 2. NLV RT-PCR results throughout study period and occurrence of illness outbreaks associated with oysters from study area.



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