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Prevalence of Norovirus in produce sold at retail in the United Kingdom

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ABSTRACT

To acquire data on contamination with Norovirus in berry fruit and salad vegetables in the United Kingdom, one thousand one hundred and fifty two samples of fresh produce sold at retail in the UK were analysed for Norovirus. Of 568 samples of lettuce, 30 (5.3%) were Norovirus-positive. Most (24/30) lettuce samples which tested positive for Norovirus were grown in the UK and 19 of those 24 samples contained NoV GI. Seven/310 (2.3%) samples of fresh raspberries were Norovirus-positive. Most (6/7) of the positively-testing fresh raspberry samples were imported, but no predominance of a genogroup, or any seasonality, was observed. Ten/274 (3.6%) samples of frozen raspberries were Norovirus-positive. The country of origin of the positively-testing frozen raspberry samples was not identified in most (7/10) instances. The collected data add to the currently limited body of prevalence information on Norovirus in fresh produce, and indicate the need for implementation of effective food safety management of foodborne viruses.

1. Introduction

Norovirus is the commonest cause of gastrointestinal disease in the U.K. and many other countries, causing millions of cases annually. Norovirus infections are a major source of disruption in communities such as schools, hospitals and nursing homes, with a significant financial burden annually (Sandmann et al., 2017). Although transmission is predominantly from person to person, consumption of contaminated foodstuffs, particularly those eaten with minimal or no prior preparation such as berry fruits and salad vegetables, has been prominently implicated in several outbreaks of Norovirus gastroenteritis globally (Advisory Committee on the Microbiological safety of Food, 2015). The European Food Safety Authority (EFSA), risk ranked the combination of leafy green vegetables, eaten raw as salads, together with Norovirus, as of 3rd highest importance for human cases of infection originating from food of non-animal origin in the European Union (EU) (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013). EFSA also ranked raspberries/Norovirus and strawberries/Norovirus as being the 4th and 5th combination respectively most often linked to foodborne human cases originating from food of non-animal origin in the EU (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013). However, the full contribution of contaminated food to the burden of disease caused by Norovirus is poorly understood, and data on contamination with Norovirus in berry fruit and salad vegetables is required to facilitate assessments of population exposure to the virus. However, despite the quite regular reports of outbreaks of Norovirus gastroenteritis implicated to consumption of fresh produce items, there is only very limited information on the prevalence of virus contamination in fresh produce (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014a; EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014b).

Currently, there are no data on prevalence of Norovirus within berry fruit and leafy green vegetables sold at retail in the UK, which is a key evidence gap regarding the extent of foodborne virus contamination in this country (Advisory Committee on the Microbiological safety of Food, 2015). To acquire representative data, this study aimed to collect and analyse samples of lettuce, fresh raspberries and frozen raspberries as key examples of leafy green vegetables and berry fruits respectively. The number of samples testing positive may indicate the level of exposure of the UK population to Norovirus from consumption of these fresh produce items.

2. Materials and methods

2.1. Survey design

The survey was performed between March 1st' 2015 and April 7th' 2016. Fresh produce samples were taken from 4 United Kingdom

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countries: England, Northern Ireland, Scotland, and Wales. The overall number of samples taken from each country were planned to be representative of each country's proportion of the UK population. Samples were taken from four regions in England (Devon, London, Manchester and Southampton), 3 regions in Scotland (Aberdeen, Dundee and Glasgow), and 2 regions each in Northern Ireland (Belfast and Londonderry) and Wales (Bangor and Cardiff). This was done to ensure samples were collected to cover the UK as proportionately as was feasible, and that samples were not predominantly taken from a localised region e.g. London and the South-East.

The samples were taken from 4 categories of outlets: Wholesalers (including suppliers of catering establishments and restaurants), Supermarkets, Markets (including farmers' markets, stalls, pick-your-own and on-line stores), and Small Retailers (e.g. convenience stores). The sampling plan attempted to reflect the higher market share that supermarkets have for food purchases in the UK (information from DEFRA's Food Statistics Pocketbook, 2014). According to this source, 82% of food purchases are made from the top 10 supermarkets. It was possible to approximate this for the English regions, since due to the large population a larger number of samples were taken. For Northern Ireland, Scotland and Wales, it was not possible to precisely reflect this proportion, but more samples were taken from supermarkets than from the other outlet types in these countries. Frozen berries were not sampled from markets as it is unlikely frozen produce will be on sale at such outlets.

No weighting was given to UK-produced versus imported produce, as the relative levels of availability of each are not known. Also, in the lack of relevant detailed information, it was assumed that per capita consumption of produce is the same for the 4 countries of the UK.

2.2. Collection of samples

Whole lettuce heads and punnets/bags of fresh/frozen raspberries were taken at randomly selected sampling points within each retail outlet. Only samples of open leafed lettuce (e.g. Cos/Romaine/Butterhead. Not Iceberg or any lettuce with a similar closed leaf appearance and not ready-to-eat bagged lettuce) were collected. Lettuces of this type were considered most likely, due to the loose nature of their leafy heads, to retain viruses that may have contaminated them at primary production. One thousand twelve hundred and eighty-nine samples were collected in total: 631 samples of lettuce, 346 samples of fresh raspberries, and 312 samples of frozen raspberries. Approximately 90 samples of fresh produce were collected each month, by SteriCycle Ltd. Immediately upon collection, samples were placed in cool boxes including ice packs and dispatched to Fera Science Ltd (Fera) by next day courier.

2.3. Sample receipt

Immediately upon receipt at Fera, all samples were examined for suitability; samples were rejected if unsuitable (i.e. wrong type of produce, sample degraded, lack of paperwork). From each accepted sample, two portions of 25–30 g were taken and immediately spiked with 10^6 genome equivalents of mengovirus (strain vMC0) as process control (Ruhanya et al., 2015). One portion was analysed immediately, the other stored (at 4 °C for lettuce and fresh raspberries and at −20 °C for frozen raspberries). Another portion of 25–30 g was taken from each sample but not spiked with mengovirus.

2.4. Sample treatment

Viruses and nucleic acids were extracted from each mengovirus-spiked sample following the method of Anonymous (2013). Briefly, virus was eluted from the food surfaces by immersion in alkaline buffer containing beef extract, then precipitated by flocculation and centrifugation. Nucleic acids were extracted from the flocculate by commercial kit (NucliSens, Biomerieux). A final volume of 100 µl nucleic acid extract was obtained from each sample.

2.5. Detection of Norovirus and mengovirus

The RT-PCR was performed using a Bio-Rad CFX96 Touch™ Real-Time PCR Detection System. Detection of Norovirus GI and GII was performed on the mengovirus-spiked sample portions following the qualitative detection protocol described in Anonymous (2013). Detection of mengovirus vMC0 was likewise performed following the protocol described in Anonymous (2013). Five microliter aliquots of both neat extract and 10^{-1} dilution were analysed by real-time RT-PCR in duplicate for Norovirus, but only the 10^{-1} dilution was analysed in duplicate for mengovirus.

2.6. Interpretation of RT-PCR results

All amplification data were converted to logarithmic plots using a Bio-Rad CFX Manager version 3.1 program, with the cycle threshold set manually. Analysis was considered to have failed if no external amplification control (EAC) signal was observed, if the recovery of the process control was less than 1%, or if RT-PCR signals were observed in the amplification negative control. If analysis of a sample failed, the stored mengovirus-spiked sample was taken for analysis. Only samples from which replicate RT-PCR results were obtained were classed as positive for the presence of Norovirus RNA. Singlicate RT-PCR sample results were not taken into account.

3. Results

In total, 1289 samples were received. Ninety samples were rejected immediately upon receipt, for several reasons including late delivery and poor condition (e.g. excessive liquid in raspberry samples). Forty seven RT-PCR based analyses for Norovirus were considered to have failed, when recovery of the sample process control virus (SPCV) was below 1% after 2 successive tests. Finally, 1152 samples were tested for Norovirus. In several samples, RT-PCR signals were observed only in one replicate assay. Replicate (either within one test or over two tests) NoV RT-PCR signals were obtained from 4.1% samples. In the early stages of the survey, if a sample portion produced an RT-PCR signal, the second portion (spiked with mengovirus) was analysed in a second test. Later in the survey however time and resource did not permit analysis of the second spiked portion(See. Table 1).

Table 2 shows the summary of results obtained from analysis of the lettuce, fresh raspberry and frozen raspberry samples. Replicate Norovirus RT-PCR signals were detected in 5.3% lettuce samples, replicate GI signals in 3.9%, replicate GII signals in 1.6%, and replicate GI/GII signals were detected together in 0.2% lettuce samples. Replicate Norovirus RT-PCR signals were detected in 2.3% fresh raspberry samples. Replicate Norovirus GI signals were detected in 1% samples, replicate GII signals in 1.6% samples, and replicate GI and GII signals were detected together in 0.3% fresh raspberry samples. Replicate Norovirus RT-PCR signals were detected in 3.6% frozen raspberry samples. Replicate Norovirus GI signals were detected in 2.9% samples,

Table 1
Summary of overall results.

Samples Received	Samples Rejected	Norovirus Analyses failed	Norovirus Analyses completed	Norovirus –positive	
1289	90	47	1152	47	
				GI	33
				GII	19
				GI & GII	5

Table 2
Summary of lettuce, fresh raspberry and frozen raspberry results.

Total samples received	Norovirus Analyses completed	Norovirus –positive	
631	568	30	
Lettuce		GI	21
		GII	9
		GI & GII	1
346	310	7	
Fresh raspberry		GI	3
		GII	5
		GI & GII	1
312	274	10	
Frozen raspberry		GI	8
		GII	5
		GI & GII	3

Table 3
Samples analysed, by origin.

Sample type	UK	Imported	Various countries[a]	Not known
Lettuce	395 (24)	145 (6)	0	28 (0)
Fresh raspberry	98 (1)	203 (6)	0	9 (0)
Frozen raspberry	67 (1)	45 (2)	52 (3)	110 (4)
Total	560 (26)	393 (14)	52 (3)	147 (4)

( ): Norovirus - positive.
[a] "Various" as stated on the item's pack. No individual country was named, and the number of countries the produce was sourced from was not stated.

replicate GII signals in 1.8% samples, and replicate GI and GII signals were detected together in 1.1% frozen raspberry samples. Table 3 shows the summary of the samples analysed, by origin. Replicate Norovirus RT-PCR signals were detected in 6.1% of UK lettuce samples, and 4.1% imported lettuce samples. Replicate Norovirus RT-PCR signals were detected in 1.0% of UK fresh raspberry samples, and 3.0% imported fresh raspberry samples. Replicate Norovirus RT-PCR signals were detected in 1.5% of UK frozen raspberry samples, 4.4% imported frozen raspberry samples, 5.8% frozen raspberry samples sourced from "various countries" (as stated on pack), and 3.6% of frozen raspberry samples of which the origin was unknown. Table 4 shows the summary of the samples analysed, by outlet type. Replicate Norovirus RT-PCR signals were detected in 13.0% of lettuce samples obtained from markets, in 5.3% of lettuce samples obtained from supermarkets, and in 5.6% of lettuce samples obtained from wholesalers. Replicate Norovirus RT-PCR signals were detected in 7.7% of fresh raspberry samples obtained from small retailers, in 1.8% of fresh raspberry samples obtained from supermarkets, and in 14.2% of fresh raspberry samples obtained from wholesalers. Replicate Norovirus RT-PCR signals were detected in 16.7% of frozen raspberry samples obtained from small retailers, and in 3.5% of frozen raspberry samples obtained from supermarkets. Table 5 shows the 30 Norovirus positive lettuce samples. Genotype 1 predominated (21 samples), and most of the GI-containing samples originated in the UK between May and August 2015 (18 samples). Table 6 shows the 7 Norovirus-positive fresh raspberry samples. No temporal pattern, or predominance of a genotype, was observed, and most (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2013) samples originated outside the UK. Table 7 shows the 10 frozen raspberry samples in which replicate RT-PCR signals were observed. As with fresh raspberries, there was no observed temporal pattern or genotype predominance. With most (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014a) of these samples, the origin could not be precisely determined.

Table 5
Norovirus-positive lettuce samples.

Date of collection	Country of origin	Norovirus
14/04/2015	UK	GII
11/05/2015	Spain	GI
11/05/2015	UK	GII
12/05/2015	UK	GII
12/05/2015	UK	GI
26/05/2015	UK	GII
08/06/2015	UK	GI
09/06/2015	UK	GI
10/06/2015	UK	GI
10/06/2015	UK	GI
15/06/2015	UK	GI
27/06/2015	UK	GI
27/07/2015	UK	GI
28/07/2015	UK	GI
28/07/2015	UK	GI
28/07/2015	UK	GI
28/07/2015	UK	GI
29/07/2015	UK	GI
29/07/2015	UK	GI
29/07/2015	UK	GI & GII
04/08/2015	UK	GI
05/08/2015	UK	GI
11/08/2015	UK	GI
23/11/2015	Spain	GII
08/12/2015	UK	GII
02/02/2016	UK	GI
10/02/2016	Spain	GI
02/03/2016	Spain	GII
07/03/2016	Spain	GII
22/03/2016	Spain	GII

Table 6
Norovirus-positive fresh raspberry samples.

Date of collection	Country of origin	Norovirus
29/04/2015	Spain	GI & GII
12/05/2015	Spain	GII
12/05/2015	Morocco	GI
30/07/2015	UK	GII
08/02/2016	Morocco	GI
07/03/2016	Spain	GII
07/03/2016	Spain	GII

4. Discussion

No previous surveys of fresh produce for Norovirus have been conducted in the UK. There have however been a limited number of surveys in other countries (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014a; EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014b). In this study, RT-PCR analysis indicated

Table 4
Samples analysed, by outlet type.

Sample type	Markets	Small retailers	Supermarkets	Wholesalers	Not identified
Lettuce	23 (3)	31 (0)	487 (26)	18 (1)	9 (0)
Fresh raspberry	12 (0)	13 (1)	276 (5)	7 (1)	2 (0)
Frozen raspberry	0	6 (1)	258 (9)	8 (0)	2 (0)
Total	35 (3)	50 (2)	1021 (40)	33 (2)	13 (0)

( ): Norovirus - positive.

Table 7
Norovirus-positive frozen raspberry samples.

Date of collection	Country of origin	Norovirus
28/04/2015	Various	GI & GII
28/04/2015	Serbia	GI
29/04/2015	Serbia	GI
29/04/2015	UK	GI & GII
05/05/2016	Various	GI
13/05/2015	NA	GI & GII
13/07/2015	NA	GII
28/07/2015	Various	GI
30/07/2015	NA	GI
09/11/2015	NA	GII

NA: Information not available.

that 30/568 (5.3%) of lettuce samples were Norovirus positive. Mattison et al. (2010) sampled 641 samples of lettuce sold in supermarkets in Canada, and found 181 positive for Norovirus (28.2%). Kokkinos et al. (2012), analysed lettuce samples at point of sale in three European countries, and found 2/149 (1.3%) samples to be Norovirus GI positive and 1/126 (0.8%) to be Norovirus GII positive. The prevalence of Norovirus in lettuce recorded in this study is intermediate between these two sets of findings. Most (24/30) lettuce samples which tested positive for Norovirus were grown in the UK. Also notable is the observation that most of the positively testing UK lettuce samples contained NoV GI, and displayed a temporal pattern in that 21 of the UK-grown samples were collected from lettuce on sale between May and August 2015. However, it cannot be determined whether this pattern is reflected in cases of Norovirus infection reported around this period, as no genotyping information is collected by UK public health bodies for sporadic cases (personal communications from various public health personnel).

Seven/310 (2.3%) of fresh raspberry samples analysed in this study were Norovirus-positive. Baert et al. (2011) found 10/150 (6.7%) samples of fresh berry fruits obtained from food companies in France were Norovirus positive, and in the study of Stals et al. (2011), 4/10 fresh raspberry samples obtained from a processing company in Belgium tested positive for Norovirus. However, when Maunula et al. (2013) analysed 60 samples of fresh raspberries at point of sale in 4 European countries, no Norovirus positive samples were identified. Most (6/7) of the positively-testing fresh raspberry samples in this study were imported from identified countries, and no predominance of a genogroup, or any temporal pattern, was observed.

In this study, RT-PCR analysis indicated that 10/274 (3.6%) of frozen raspberry samples were Norovirus positive. Maunula et al. (2013) examined 39 frozen raspberry samples from point of sale, but no Norovirus positive samples were identified. Sarvikivi et al. (2012) analysed 14 samples of frozen raspberries implicated in an outbreak in Finland and detected Norovirus in 2 (14.3%) samples, and Mäde et al. (2013) analysed 11 samples of frozen raspberries implicated in an outbreak in Germany and found 7 (63.6%) to be positive. In this study, the country of origin of the positively-testing frozen raspberry samples was not identified in most (7/10) instances. In three samples, the raspberries had been grown in several unstated countries, and in 4 samples no information was available on the origin of the fruit. This likely reflects the nature of the supply chain of this commodity, with products being sourced from various locations.

The relative numbers of samples in each UK country in this study closely reflect the relative proportions of the UK population. Similarly, the preponderance of samples from supermarkets reflects the relative market share (82%; information from DEFRA's Food Statistics Pocketbook, 2014) from of this type of outlet compared to markets, small retailers and wholesalers. The samples may thus be regarded as representative as possible of the consumption of the produce types in the UK at the time of the study, given the available information.

A rigorous interpretation of the RT-PCR data, reducing uncertainty due to any possible cross-contamination with EACs, was performed by classifying only those samples which yielded replicate RT-PCR signals, as Norovirus-positive. The EACs prescribed in ISO 15216 to be used in the RT-PCR sample analysis are derived from actual Norovirus sequences, and the primers used for amplification of the EACs are the same as those which were used to amplify the sequences from Norovirus strains. Likewise, the same probe is used for detection of the amplicons of the EAC and amplicons of any Norovirus strains extracted from the sample. Thus, the EAC RT-PCR signals produced by the GI and GII EACs are identical to the signals which are produced by amplification of Norovirus sequences from actual viruses. The EAC amplicons might be distinguished from Norovirus GI amplicons by sequencing, but in no instance when sequencing of the Norovirus amplicons was performed was confirmation obtained that they were derived from actual viruses and not from contaminating EAC. It is difficult to obtain sequence information from such small (~90 bp) nucleotide fragments. Baert et al. and Stals et al. (Baert et al., 2011; Stals et al., 2011) also reported that they were unable to obtain sequence information for the majority of Norovirus amplicons they obtained in their studies. The EACs were first described in Le Guyader et al. (Le Guyader et al., 2009), who inserted a sequence facilitating recognition by the BamHI restriction enzyme into the EAC sequence. Thus it should be possible to distinguish true Norovirus signals from contaminating EAC signals by cloning the amplicons and digestion with BamHI, but time and resource did not allow this within the study.

RT-PCR detection of Norovirus does not indicate *per se* that the virus was infectious. Nine Norovirus positive samples were selected for capsid integrity tests following the method of Topping et al. (2009), which might have allowed more information on potential infectivity to be acquired, but the results of the tests were inconclusive (data not shown). However, seven samples produced Norovirus RT-PCR signals during this test, which may be taken as further confirmation of the original result, and strongly indicative of the presence of Norovirus in these samples.

Until more prevalence data is acquired by further national or international studies, it is difficult to comment on how UK Norovirus prevalence in fresh produce, as indicated by the results of this study, precisely compares with the global situation. To date no Norovirus outbreak in the UK has been positively implicated to fresh produce contamination, and there is no routine surveillance system in place in the UK to monitor virus contamination of foods; however, some information is available from other countries. Norovirus-contaminated lettuce has caused several disease outbreaks in Europe (Ethelberg et al., 2010; Mesquita and Nascimento, 2009; Müller et al., 2016) and the USA (information from the Centers for Disease Control and Prevention "FOOD Tool" https://www.cdc.gov/foodborneoutbreaks/). Raspberries, very often imported, have been the vehicle for several outbreaks of Norovirus gastroenteritis (Einöder-Moreno. et al., 2016; Müller et al., 2015; Sarvikivi et al., 2012), and Norovirus-contaminated raspberries, particularly frozen fruits, are regularly reported in the European Rapid Alert System for Food and Feed Portal (http://ec.europa.eu/food/safety/rasff). Most of the Norovirus-positive raspberries identified in the current study were imported into the UK. With the Norovirus-positive lettuce samples, the picture is different. The majority of these were produced in the UK, within a definite time period, and most were contaminated with the same genotype (GI). The major routes whereby Norovirus gets from the intestines of infected persons to fresh produce are handlers' hands, and contaminated water used for irrigation, pesticide application or washing (Bouwknegt et al., 2015; Kokkinos et al., 2016; Maunula et al., 2013).

Virus contamination raises the question of whether food safety management systems are being effectively or thoroughly implemented in the food supply chains involved. One should not expect to find a Norovirus in one's lettuce or raspberry, and when the virus is detected it is clear that guidance such as that available from the Codex Alimentarius Commission (CAC (Codex Alimentarius Commission),

2012) is not being adhered to, and that in consequence the pathogen is entering the food supply chain with a potential to impact upon public health. To facilitate monitoring and control of foodborne viruses it may be prudent to consider a Process Hygiene Criterion for Norovirus in fresh produce. The European Food Safety Authority has stated (EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014a; EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2014b) that there is currently insufficient data on virus prevalence to establish such criteria, and has strongly recommended that appropriate data should be collected. The data from the current study confirm the vulnerability of fresh produce to Norovirus contamination, and add to the body of prevalence data which should eventually encourage the implementation of effective food safety management of foodborne viruses.

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