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Norovirus Attribution Study: Detection of norovirus from the commercial food preparation environment in outbreak and non-outbreak premises

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Abstract

Aims: Norovirus remains the most significant virological risk that is transmitted via food and the environment to cause acute gastroenteritis. This study aimed to investigate the hypothesis that the contamination of the commercial food production environment with norovirus will be higher in premises that have recently reported a foodborne norovirus outbreak than those that have not.

Methods: Sampling of commercial food production environments was carried out across a 16-month period between January 2015 and April 2016 in the South East and the North West of England by local authority environmental health departments as part of routine surveillance visits to premises. A total of 2,982 samples, 2,038 virological and 944 bacteriological, were collected from 256 premises. Sixteen of these premises, six from South East and ten from North West England, were sampled as part of a public health outbreak investigation.

Results & Conclusions: Overall, 2,038 swabs were submitted for norovirus testing, with an average of eight swabs per premises (range 4 to 23) and a median of seven. Of the premises sampled, 11.7 % (30/256) yielded at least one norovirus positive sample (environmental, and/or food handler hand swab), and 2.5 % of the swabs were positive for norovirus. A peak in the positivity rate was seen in the South East in April 2016. No associations were found between norovirus positivity and bacteriology indicators, or between bacteriology indicators and hygiene ratings.

Significance and impact of study: This study demonstrates that food premises and food handlers remain a potential source of norovirus transmission and outbreaks.

Keywords

Food, Molecular epidemiology, Viruses, Environmental, PCR (polymerase chain reaction)

Introduction

Food-related illness is a major burden on human health worldwide. According to World Health Organisation (WHO), estimates of the global burden of foodborne illness was associated with 600 million illnesses and 420,000 deaths in 2010 (Havelaar et al. 2015). Although acute gastroenteritis is varied and complex in aetiology (WHO 2015), virus infection is an important cause (Iturriza-Gomara et al. 2016), and among the enteric viruses commonly associated with transmission via food, norovirus is the most significant (Kirk et al. 2015). Data from a recent reanalysis of the Second Infectious Intestinal Disease study estimates that there are almost four million norovirus infections in the UK population annually (Harris et al. 2017).

Norovirus gastroenteritis presents as a sudden onset of diarrhoea and vomiting, usually of 2-4 days duration (Graham et al. 1994; Lopman et al. 2004; Murata et al. 2007; Rockx et al. 2002). Virus shedding can begin 3-14 hours prior to onset of symptoms and can continue for 2-5 days after infection (Atmar et al. 2008; Graham et al. 1994; Kirby et al. 2014). Shedding of norovirus particles by infected individuals has been demonstrated both in symptomatic and asymptomatic cases (Akihara et al. 2005; Huynen et al. 2013; Krumkamp et al. 2015), and, whilst asymptomatic individuals may shed lower levels of norovirus (Bernstein et al. 2015), both groups can transmit the virus.

Norovirus transmission occurs via the faecal-oral route and this, combined with the stability of the virus particle, means transmission via faecal contamination of food commodities and the environment both pose significant risks as a source of infection. The ability of the virus to survive on surfaces as a fomite has been shown within both care home (Wu et al. 2005) and hospital (Nenonen et al. 2014) settings. Furthermore, *in vitro* survival experiments have demonstrated the recovery of norovirus from fomites after prolonged periods, suggesting that environmental sources remain a stable reservoir of virus (Lamhoujeb et al. 2009; Liu et al. 2009).

Food matrices and water sources have been shown as transmission vehicles (Hardstaff et al. 2018), as well as transmission linked to contact with contaminated environmental sources such as food preparation areas (Seitz et al. 2011). Agricultural workers can be a direct source of contamination and

cross-contamination of ready-to-eat salad vegetables, with Leon-Felix and colleagues (2010) demonstrating the contamination of peppers in the field and on the hands of pickers, classifiers and packers. Similarly, food handlers pose a risk in the food preparation environment, and are advised to have a 48-hour exclusion from work following an episode of gastrointestinal infection (Food Standards Agency 2009), and this exclusion should be combined with ongoing good hygiene practices and hand washing to prevent contamination. The presence of norovirus on the hands of food handlers means that it can easily be transferred to utensils; work surfaces and food; between utensils, work surfaces and foods once they themselves are contaminated as shown in a number of published studies (Hardstaff et al. 2018; Ronnqvist et al. 2014; Sharps et al. 2012; Stals et al. 2013; Tuladhar et al. 2013; Verhaelen et al. 2013). Standardised methods have been validated for detection of norovirus in some foods and on contact surfaces (Anonymous 2007), and such molecular detection-based approaches have been shown to be useful in interrogating environmental contamination during norovirus outbreaks (Park et al. 2015).

Environmental transmission of norovirus through poor hygiene practices means that it is likely that the virus is transferred alongside other organisms found in vomitus and faeces. Alongside this, limitations in laboratory detection of low levels of norovirus transferred on to surfaces mean that environmental contamination may be overlooked. Bacterial indicators have been established as a proxy to highlight poor hygiene practices (Willis et al. 2012). The presence of indicator bacteria in environmental samples, although not an inherent hazard, can indicate poor practice in food premises. Four key indicators are used routinely; *Escherichia coli*, Enterobacteriaceae, coagulase positive staphylococci (including *Staphylococcus aureus*) and the total aerobic colony count (ACC)) (Greenwood 2007a; Greenwood 2007b; Health Protection Agency 2009). The bacterium *E. coli* is predominantly found in the gastrointestinal tract of humans and animals and is used as an indicator of faecal contamination. The Enterobacteriaceae family is a group of bacteria that are used to assess general hygiene and includes *E. coli* and *Salmonella* species alongside further species of bacteria that are found in the intestinal tract of humans and animals, or those found on the surfaces of plants and other environmental sources. The presence of coagulase positive staphylococci in the food processing environment is likely to be associated with poor hand hygiene and failure to adequately clean hand contact surfaces, although contaminated products of animal origin should also be considered as a source. Aerobic colony counts

are more difficult to interpret in terms of risk to the general public than for other indicator organisms but do represent a general indicator for the efficacy of cleaning practices (Health Protection Agency 2009). All bacterial contaminants are readily removed through effective disinfection and cleaning therefore, can be used to determine the efficacy of these practices in premises.

This study aimed to investigate the hypothesis that the contamination of the commercial food production environment with norovirus will be higher in premises that have recently reported a foodborne norovirus outbreak than those that have not. It was expected that the levels of environmental contamination were likely to be seasonal, with greater levels of contamination being detected in the winter months (November to March).

Materials and Methods

A prevalence survey was carried out in food premises across the North West (NW) and South East (SE) of England. Food preparation premises were defined as a commercial or voluntary organisation that prepared and served food to the final consumer. This included restaurants, public houses, cafés, takeaways, hotels and guest houses, and caterers, but excluded passenger-carrying ships that travel outside the UK, private houses, mobile retailers, manufacturers and suppliers.

Surveillance sampling

It was estimated that sampling 250 premises would allow detection of environmental contamination in the commercial food production environment at a level of 20.0 %, with 95.0 % confidence and an error in the final prevalence estimate of ±5.0 %.

Sampling was carried out by local authority (LA) Environmental Health Officers (EHOs) undertaking routine inspections of catering premises. In the SE, samples were collected by: London Borough of City of London; London Borough of Ealing; Royal London Borough of Greenwich; London Borough of Hounslow; London Borough of Southwark; London Borough of Sutton; London Borough of Tower Hamlets; London Borough of Wandsworth; Hertsmere Borough Council. In the NW, the LAs involved in sampling were: Allendale Borough Council; Blackburn with Darwen Borough Council; Bolton Council; Bury Council; Cheshire East Council; Eden Borough Council; Liverpool City Council; Preston City Council; Ribble Valley Borough Council; Salford City Council; Sefton Council; Stockport Council; Wyre Borough Council.

Sample collection was carried out at monthly intervals and covered a 16-month period between January 2015 and April 2016. Premises were selected at random to represent both the Food Hygiene Rating Scheme (FHRS) scores [where business are scored from 0 - urgent improvement is required, to 5 - hygiene standards are very good] (Food Standards Agency 2019a), and the type of premises that were representative in the specific geographical areas. The FHRS score was determined either at the time of the sampling visit or was an existing score that had been allocated to the premises. Inspections were recorded using the current UK Food Surveillance System (UKFSS; Food Standards Agency 2019b), as

undertaken routinely by LAs. During inspection visits, EHOs took two types of swabs from food and hand contact surfaces: bacteriological swabs, to allow testing for bacterial indicators of hygiene, using SpongeSicle™ swabs (3M); and virology swabs, for the detection of norovirus RNA, using viscose swabs premoistened with deionised water (Technical Service Consultants Ltd.). In food preparation environment areas, sampling was prioritised in higher-risk hand contact areas, including; refrigerator door handle, food preparation surfaces (e.g., chopping board), and kitchen sink. The toilets used by members of staff were also sampled, through collection of swab samples from the inside of the door handle into the toilet, and the toilet flush handle. A standardised questionnaire was completed by EHOs that recorded details on the type of premises (cafe, restaurant, pub, gastro-pub, takeaway, hotel etc.), number of covers (if applicable), number of staff, number of sinks and wash basins for handwashing in the food preparation environment, number of toilets designated for staff use, type of hand drier.

Sampling during outbreaks

The prevalence survey was supplemented by including premises that were being investigated because of a suspected foodborne norovirus outbreak. An outbreak was defined as either: (a) two or more people from more than one household who are thought to have a common exposure to proven norovirus infection; or (b) clinically, based on the Kaplan criteria (Kaplan et al. 1982).

Foodborne norovirus outbreaks were defined according to the following criteria:

- The outbreak was a point source outbreak;
- There was a common food exposure – meal/buffet lunch/wedding breakfast;
- Foodborne transmission was the only or predominant transmission pathway identified by investigators;
- The cases did not have any other common exposure that could explain the outbreak apart from the consumption of food;
- The outbreak was not known to be the result of a guest or member of staff vomiting in a public area.

Where possible, in addition to environmental swabs at outbreak premises, stool samples from members of staff were collected via the appropriate Environmental Health Department and clinical specimens

associated with outbreaks of norovirus in food premises in the NW and SE were submitted to the respective lead Public Health Laboratory for norovirus detection.

Virology: Norovirus testing

Upon arrival, viscose swabs were immersed in lysis buffer (L6, Severn Biotech). Total nucleic acid was extracted from the entire sample (spiked with an exogenous internal control (IC) mengovirus MC_0) (Costafreda et al. 2006) using GTC-silica based method based on that described by Boom et al. (1990). RNA was converted to cDNA in a random-primed reverse transcription reaction, and the presence of genogroup I (GI) and genogroup II (GII) norovirus detected by real-time PCR as previously described (Kageyama et al. 2003). Results were interpreted qualitatively only, with samples with cycle threshold (Ct) values below 40 being considered positive. Samples with Ct>40 were considered negative only if the IC RT-PCR results were within the expected Ct range. Samples with Ct>40 in which IC RT-PCR results were not within the expected Ct range were retested in a ten-fold dilution from the RNA extract to compensate for potential inhibition. If the sample remained inhibitory in the dilution, the results were reported as an invalid test.

Bacteriological testing

SpongeSicle™ (3M) swabs with 10 ml neutralising buffer were supplied by the PHE Food, Water and Environmental Microbiology Services (FWEMS) for LAs to collect bacteriological swabs. Sampling Officers could collect samples from a defined template area, usually 10 cm by 10 cm, or from a random area at the sampling point and method of sampling recorded on the sample request form submitted with the samples.

Samples were tested based on FWEMS standardised methods [Method F10 (ACC), F12 (*Staphylococcus*), F20 (*E. coli*) and F23 (Enterobacteriaceae)] with samples being diluted 1:100 in buffered peptone water prior to direct plating onto appropriate selective methods. For random or non-template swabs, results were provided as colony forming units (cfu) per swab or per cm^2, respectively. Samples were enumerated for: coagulase positive Staphylococci, including *Staphylococcus aureus;* β-glucoronidase *Escherichia coli;* Enterobacteriaceae. For template area swabs (10 x 10 cm), results

were represented as cfu per cm^2. Samples were enumerated for: total aerobic colony count (ACC); coagulase positive Staphylococci, including *Staphylococcus aureus;* β-glucoronidase *E. coli;* Enterobacteriaceae.

Results for bacteriological swabs were interpreted depending on whether they were sampled from a measured area or from a random area on a surface, adapted from Willis et al. (2012). Briefly, for a measured area swab, ACC results <100 cfu per cm^2 were considered satisfactory, results of ≥100 to <1,000 cfu per cm^2 were considered of borderline microbiological quality and results of ≥1000 were considered unsatisfactory. For coagulase positive Staphylococci, β-glucoronidase *E. coli and* Enterobacteriaceae a satisfactory result was <2 cfu per cm^2 and an unsatisfactory result was ≥2 cfu per cm^2. For random area swab, a satisfactory result was <200 cfu per swab and an unsatisfactory result was ≥200 cfu per swab.

Stats and data analysis

The R software environment (v 4.0.5) was used for data reformatting and statistical analyses (R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.).

The following package was used within the R software environment: tidyverse (Wickham et al. 2019).

Results

A total of 256 premises were sampled across 16 months in two regions of England; 124 in SE and 132 in NW. Of these, 240 were sampled for surveillance and 16 as part of an outbreak investigation. A total of 2982 swabs were collected; 2038 for norovirus detection and 944 for bacteriological indicators (Figure 1 & Table 1).

Bacteriology

No significant associations were identified between bacteriological indicators and norovirus positivity (Table 1) ($p > 0.05$, Chi Squared Test); however, we note that *Enterobacteriaceae* were the most commonly isolated bacterial indicator (Not significant, $p = 0.869$, Chi-squared test). Swabs were collected from premises representing the six FHRS scores, but no significant association was identified between the FHRS score and the isolation of bacterial indicators at unsatisfactory levels ($p > 0.05$, Chi Squared Test).

Virology

Among the premises surveyed, norovirus RNA was detected in at least one environmental swab at 7.0 % (18/256), or at least one staff hand swab at 3.0 % (8/256), or at least one environmental and hand swab at 1.6 % (4/256). Overall, norovirus RNA was detected in 2.3 % (35/1493) of environmental swabs and 3.0 % (15/501) of staff hand swabs collected (Figure 1). More norovirus RNA positive results were obtained from samples collected in the SE compared to the NW ($p < 0.05$, Chi-squared Test).

We compared detection of norovirus RNA in premises associated with an outbreak with those sampled for routine surveillance purposes (Figure 1) and detected it in 25.0 % (4/16) of outbreak premises and 10.8 % (26/240) of surveillance premises ($p = 0.192$, Chi-squared test with Yates correction). This represented 3.3 % (6/184) swabs collected at outbreak premises and 2.4 % (44/1810) swabs from surveillance premises. There was no significant association between norovirus RNA positive premises identified through sampling performed in response to an outbreak compared to when it was performed for routine surveillance ($p = 0.457$, Fisher's exact test).

Samples were collected from premises in both regions that represented all six FHRS categories, however 0 ratings were underrepresented in the NW. Norovirus RNA positive swabs were found in similar proportions in premises across the FHRS categories from 0 to 5, with no statistical significance when individual FHRS values were compared to the others ($p > 0.05$, two proportion z-test). Norovirus RNA positive premises in the NW were grouped to the higher FHRS categories of 3, 4 and 5, whereas in the SE positive results were obtained across the six FHRS categories of premises (Figure 2A).

As a proxy for the size of premises, the number of covers and the number of kitchen staff were compared against the number of norovirus RNA positive premises and swabs. Most premises where norovirus RNA was detected served fewer than 100 covers or had five or less kitchen staff (Figure 2B and 2C). The mean and median number of staff per norovirus positive premise were 4.4 and 2 (range 1-9) respectively in the NW and 3.3 and 2 (range 1-15), respectively in the SE compared to 5.6 and 3 (range 1-70) in the NW, and 4.8 and 4 (range 1-28) in the SE among the norovirus negative premises.. A significant association was found in norovirus RNA positive premises relating to the number of kitchen staff in the South East, where under four ($p = 0.027$, Fisher's exact test) and under three ($p = 0.005$, Fisher's exact test) were found to be associated with detection of norovirus compared to higher numbers of kitchen staff. The number of covers recorded by premises was not associated with a positive norovirus RNA result ($p > 0.05$, Fisher's exact test).

We looked at the availability of dedicated staff toilets (Figure 2D), hand washing (Figure 2E) and drying facilities (Figure 2F) compared against the number of norovirus RNA positive premises and swabs. It was noteworthy that 29.0 % (74/256) premises were reported to have no dedicated staff toilet facility. Where premises had one or no dedicated staff toilet there was no significant association with norovirus positivity of the premises ($p = 0.184$, Fisher's exact test).

Wash hand basin (WHB) and hand drying facilities in the kitchen were reviewed (Figure 2E-F), with norovirus RNA positive results being higher where only one WHB is recorded ($p = 0.492$, Fisher's exact test). Hand drying method was usually recorded as paper towel, although cloth towels and air hand dryers were also noted to be in use on premises. No association was found between paper or cloth towel compared to air drying for norovirus RNA positive premises ($p = 0.264$, Fisher's exact test).

The survey captured six key groups of premises type (Figure 3). Despite being represented more frequently in the survey, public houses and cafés were not statistically associated with norovirus RNA positivity ($p = 0.132$, Chi-squared test).

The positivity and date of collection were evaluated. There was a month on month increase in the number of positive swabs in months 14 to 16 of the study (Figure 4). There was a significant increase in the proportion of positive swabs in April 2016 compared to the previous 15 months of the study ($p < 0.001$, both Chi-squared Test with Yates correction, or Fisher's exact test). We confirmed that this was not associated with an artefact of sampling activity ($p > 0.05$, pairwise two-proportion z-test).

Discussion

We present data from a longitudinal study to assess environmental norovirus contamination in food premises in England, including samples collected directly from food handlers' hands. Overall, 11.7 % (30/256) of the premises sampled yielded at least one norovirus positive sample (environmental, and/or hand swab), and 2.7 % of the swabs were positive for norovirus in total. The proportion of premises in which norovirus was detected in the SE was 21.7 % compared to 2.3 % in the NW.

Differences in the proportion of positive samples between the SE and the NW were not associated with testing protocols or laboratory differences, as demonstrated in a head-to-head comparison of methodologies (personal communication), and no differences were seen between the number of staff between norovirus-positive and -negative premises or between regions. It is possible that observed differences may be associated with local differences in norovirus epidemiology at any given time, or that population density could play a role in norovirus prevalence, whereby densely populated areas such as the SE experience higher case rates than the more sparsely populated areas sampled in the NW. However, national surveillance data, which are biased primarily towards capturing health and social care associated outbreaks, do not allow us to explore this further at present. Comparison of these data with a similar study conducted in The Netherlands, show that Boxman et al (2011) also found no association between number of staff and norovirus detection rates, but did identify a positive association with population density and norovirus detection rate in the environment.

Further, the proportion of premises reporting at least one norovirus-positive environmental and/or hand sample in this study was higher than reported by Boxman et al. (2011): 11.7 % *vs* 4.2 % of establishments and 2.7 % *vs* 1.7 % of the environmental samples. However, the proportion of norovirus positive premises during outbreak investigation was not significantly different to that identified in routine surveillance sampling (25.0 % *vs* 10.8 %), which is contrary to findings from the study in The Netherlands, which identified norovirus positive samples in 61.1 % of premises associated with outbreaks of gastroenteritis and in 4.2 % that had no outbreak link (Boxman et al. 2011). One major difference between our study and that of Boxman et al. (2011) is that we specifically excluded premises in health and social care institutions, given that they constitute a population at high risk of norovirus

infection and outbreaks are not representative of the general population. This difference may also account at least in part for this discrepancy and the availability of a larger number of premises associated with norovirus outbreaks in the Boxman et al. (2011) study.

More than half of premises sampled had a FHRS score of 5. Whilst the selection of premises based on FHRS score was designed to be cross-sectional across the ratings, in practice this was influenced by human factors in selection of individual premises by EHOs, as well as availability and accessibility to individual businesses on the day of sampling. Whilst the number of premises sampled from each FHRS score is not equally distributed, data analysis shows at four of the SE premises norovirus RNA was detected in at least one food handler and one environmental swab. Of these, two premises were FHRS score 5, one score 3 and one score 2. No premises in the NW had samples where norovirus RNA was detected in both food handler and environmental swabs. The FHRS score is a complex and multicomponent tool that provides a snapshot of a business' compliance with food hygiene law (Food Standards Agency, 2019a).

Bacterial indicators have been used as one of the measures of a business' food hygiene status and are used by EHOs in the assessment of compliance to support the allocation of a FHRS score. This study was the first attempt to link FHRS score with virological testing, and these observations highlight the complexities of understanding the detection of viral RNA in a catering environment. In this study, bacterial indicators did not provide an effective indicator for norovirus. A four-fold higher number of premises had bacterial indicator organisms isolated (118) compared to those with a norovirus being detected (30). There has been a tradition of using the presence of bacteria as a proxy for presence of viruses, however the correlation between the results for these two very different organisms has been an issue of longstanding debate, with Lees (2000) highlighting that *E. coli* is not an appropriate indicator for viral contamination in bivalve shellfish. This is combined with direct evidence from outbreak settings that the presence of bacteria in samples does not correlate with the presence of viral pathogens (Koopmans and Duizer 2004).

In one outbreak investigated as part of this study, norovirus-positive samples were identified from affected consumers, a food handler and an environmental site. Although no virus sequence was

obtained from the single environmental positive swab associated with this outbreak, further epidemiological investigation determined prior illness of the food handler as the most plausible source of the contamination which led to the outbreak (Smith et al. 2017). Across the study, 30.0 % of norovirus positive swab samples were from food handler hands and 70.0 % were from other environmental sources. Overall, the food handler hand swabs had a positivity rate of 3.0 % and the other environmental sampling points had a positivity rate of 2.0 % and evidence exists of transmission of contamination between surfaces and food and between contaminated hands and food and surfaces (Rönnqvist et al. 2014; Sharps et al. 2012; Stals, 2013).

Food handlers remain a potential source of norovirus transmission associated with outbreaks. Handwashing is a key control factor in the spread of gastrointestinal infections, including norovirus. Within a recent social sciences study, the adequacy and frequency of handwashing was reported as being highly variable and was identified as a knowledge gap for food handlers (FSA 2017). Asymptomatic food handlers have been implicated as a source of food-borne illness, with two individuals involved in food preparation and service being reported as the source for an outbreak in Taiwan (Chen et al. 2016) and nine food handlers in China (Xue et al 2014). In a report from an outbreak on a cruise ship, handwashing before meals was found to be protective for passengers, highlighting its importance as a control measure (Chimonas et al. 2008). This has been demonstrated in simulation experiments for sandwich production. Stals et al. (2015) showed a reduction in norovirus from all reservoirs where there was high compliance of handwashing after visiting the toilet. Overall, washing hands after visiting the toilet, wearing gloves, disinfection of hands and surface disinfection was an effective way to prevent norovirus contamination of food. Derrick et al. (2021) identified in a simulation experiment that norovirus is transferred from food handler's gloved hands to food and to the environment demonstrating that hand hygiene is a critical point for norovirus control.

In this study we cannot rule out onward transmission in those establishments that were not associated with a reported outbreak. The origin of the contamination can therefore be difficult to ascertain, particularly in the absence of sequence information. The ability to genotype norovirus positive samples with low viral loads is the main limitation to linking environmental samples with cases in suspected foodborne outbreaks. Although this is less of a challenge when clinical samples are available, methods

based on PCR and Sanger sequencing are not always successful and may have some inherent biases. The use of massively parallel sequencing methods for outbreak investigation may offer increased success for sequencing norovirus from environmental samples, and higher resolution data to resolve linkage of transmission events (Wong et al. 2013; Silva et al. 2021).

This study has various limitations. The overall prevalence of norovirus in the food premises was lower than the 20.0 % of premises expected to be positive in our sample size estimations. Therefore, this study was insufficiently powered to prove or disprove some of our hypotheses. It must be taken into consideration that norovirus is a seasonal infection, and that there is wide year-to-year variation in the overall prevalence of norovirus disease. Norovirus surveillance data collected by PHE demonstrated low levels of norovirus disease and norovirus outbreaks in England during the study period (Public Health England, 2019), therefore the results of this study must be interpreted in the context of a low incidence of norovirus illness in England. It is likely that at times of higher norovirus circulation, and more frequent outbreaks, differences between premises associated with outbreaks and those that were not may become more evident than in a season with a low base line level. In order to assess seasonal variations in the rates of norovirus positivity of food premises, longer term and sustained surveillance is necessary to overcome biases introduced by the natural year-to-year variations seen in norovirus circulation in the population.

We were unable to demonstrate a clear seasonal distribution in positivity rates during this study. We did, nevertheless, observe a significant increase in the rate of positive swabs detected during the last month of the study (April 2016), and this coincided with an increase in the overall reporting of norovirus outbreaks to the national surveillance system (Public Health England, 2019). Extension of the sampling period would have been needed in order to confirm attribution of this increase to the seasonal variation in incidence of norovirus infections in the population. Further, there is recognition of under-reporting of norovirus outbreaks associated with food premises, as demonstrated in a capture-recapture analysis of seafood-associated norovirus outbreaks, with evidence of under-reporting across three organisations involved in monitoring and investigating food-associated norovirus outbreaks (Hardstaff et al. 2018).

Finally, during the study we attempted to characterise norovirus genotypes from RNA-positive environmental and hand swab samples. However, viral loads were too low to be able to recover sufficient RNA for sequencing-based analyses. Methodological improvements are needed to improve capability for sequencing viral RNA from low copy and / or samples of lower integrity, which are often characteristic of environmental samples collected during outbreak investigations. For example, during the study, an outbreak of gastroenteritis at a wedding was investigated in which we were able to characterise the outbreak strain as a GI.6 norovirus from stool samples collected from guests and food-handlers, but we were unable to further characterise the virus from environmental or food samples (Smith et al. 2017). Improvements in sequencing capabilities would facilitate linking human cases with environmental samples to elucidate transmission pathways more clearly, which, in turn, would identify high-risk sites in catering premises to help inform infection control practices.

These limitations are in practice difficult to overcome and will require innovative approaches to future surveillance. For example, systems for pathogen surveillance linked to environmental epidemiology is a fast-developing area in the science of public health: this approach has been informative during the COVID-19 pandemic (Farkas et al. 2020), and as methodologies and implementation are being developed for post-pandemic surveillance, norovirus must be considered a priority pathogen in such systems. Further, to complement population approaches such as wastewater sampling, these need to be combined with longitudinal sampling studies in catering premises to allow better understanding of transfer of norovirus within food preparation environments and transfer to humans from environmental reservoirs. These could also be paired with self-reporting and social media data, which can be useful for data capture in well-defined populations that can be traced and linked to food consumption behaviours. Indeed, during the COVID pandemic, changes in behaviour linked to messaging around personal hygiene practices and leading to individuals taking action to exclude themselves from social contacts if symptomatic or based on self-testing may have longer-term impact for the food industry, which may be even more important as the catering industry recovers, and as there may potentially be a more susceptible population post-pandemic, especially if a novel norovirus variant displaces the previously dominant GII.4/2012 (Douglas et al. 2021; O'Reilly et al. 2021).

Norovirus as a major aetiological agent of gastroenteritis for which food and environmental transmission are major pathways (Kirk et al. 2015), there is a need for investment in innovative surveillance systems

to develop evidence-based guidelines, inform infection control-related policies and practices, outbreak response and underpin interpretation of norovirus testing results from environmental and food samples.

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Conflict of Interest

None

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Table and figure legends

Figure 1: Schematic detailing the food business survey undertaken. During the survey 2982 swabs (blue pathway) were collected from 256 premises (orange pathway) which were recruited either through routine surveillance (green pathway) or in response to an outbreak report (yellow pathway). Asterisk indicates where the analysis excludes 44 swab samples: 30 environment and 5 hand swabs with inhibitory results; 6 environment and 3 hand swabs where the premises at which they were collected were not recorded as outbreak or surveillance.

Figure 2: Summary of results from the food business survey. Figures show the number of premises where norovirus RNA was detected on food handler's hands (green), or an environmental swab (gold) or both (pink), according to the premises' (A) food hygiene rating score (FHRS); (B) number of covers; (C) number of kitchen staff; (D) number of dedicated staff toilets; (E) number of hand wash basins; and (F) type of hand drying methods available. NR = not recorded.

Figure 3: Distribution of types of premises sampled in the North West and South East regions classified by premises type (coloured as shown in the legend) during the NoVAS study by sample date (x-axis, week number-year [WW-YYYY]), and showing the number of premises sampled (left y-axis, bars) and data on the number of positive environmental swabs (left y-axis, dotted line) and the number of positive hand swabs (left y-axis, dashed line). Data are arrayed on the right y-axis by Food Hygiene Rating Scheme score, 0-5, or if not recorded (NR).

Figure 4: Distribution of premises sampled in the North West and South East regions classified as outbreak (yellow bars), surevillance (gren bars) or not recorded (blue bars) during the NoVAS study by sample date (x-axis, week number-year [WW-YYYY]), and showing the number of premises sampled (left y-axis, bars) and data on the number of positive environmental swabs (left y-axis, dotted line) and the number of positive hand swabs (left y-axis, dashed line). Data are arrayed on the right y-axis by Food Hygiene Rating Scheme score, 0-5, or if not recorded (NR).

Table 1: Characteristics and number of premises sampled, norovirus positivity and unsatisfactory bacterial indictor organism results.

Supplementary information

Table S1. Distribution of the premises by food region, samples by food region and norovirus positive swabs by Food Hygiene Rating Scheme score.

Table S2. Type of premises sampled and number that had norovirus detected

Figure 1

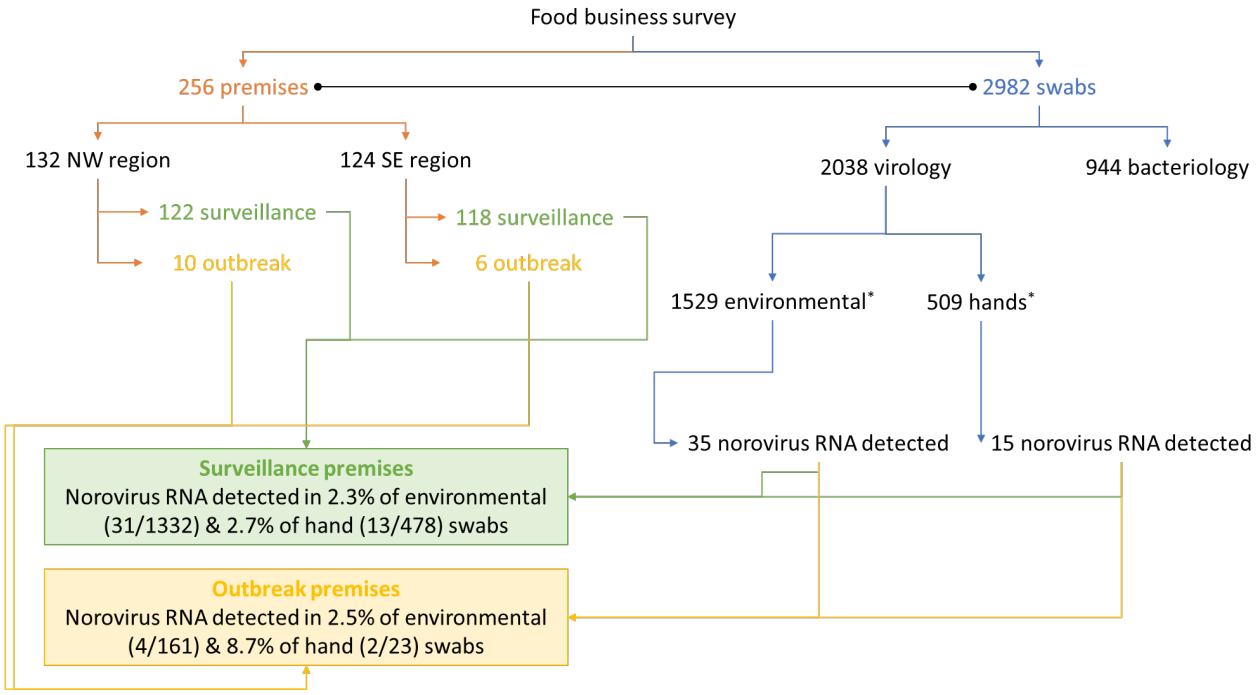

Figure 2

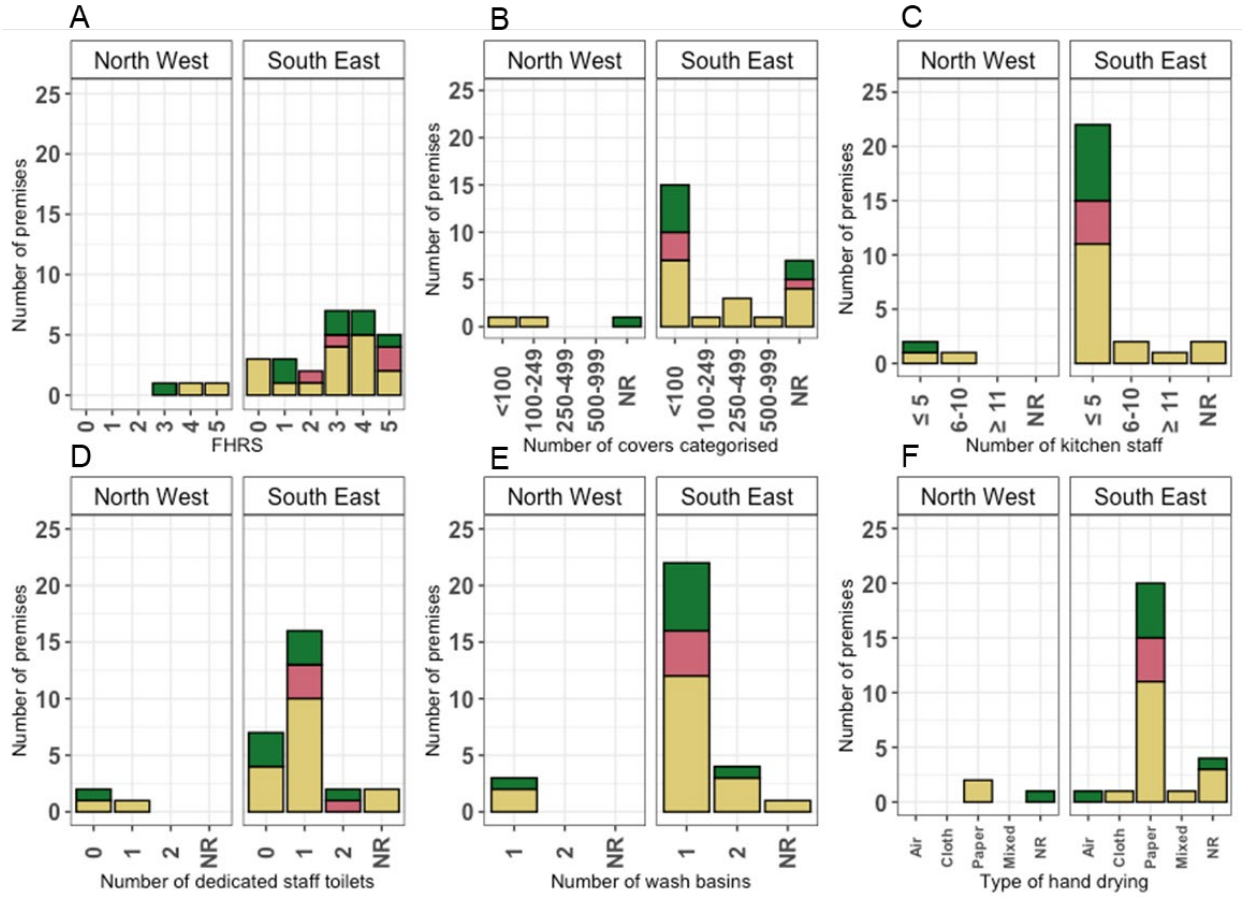

Figure 3

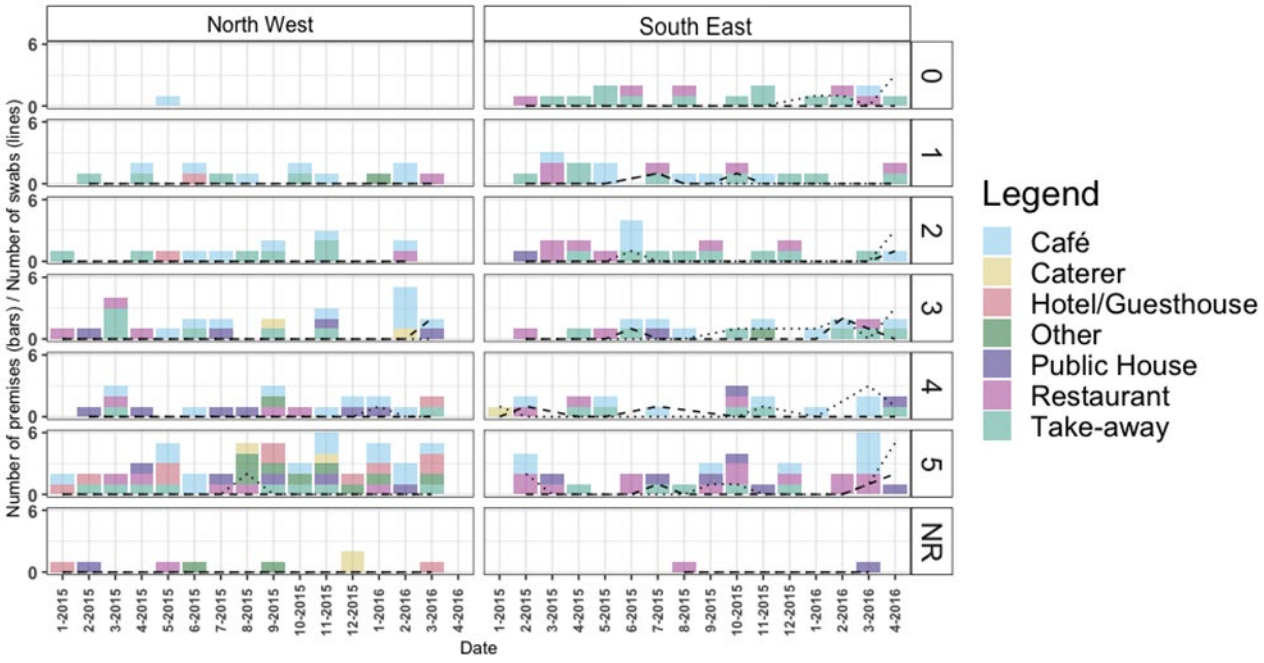

Figure 4

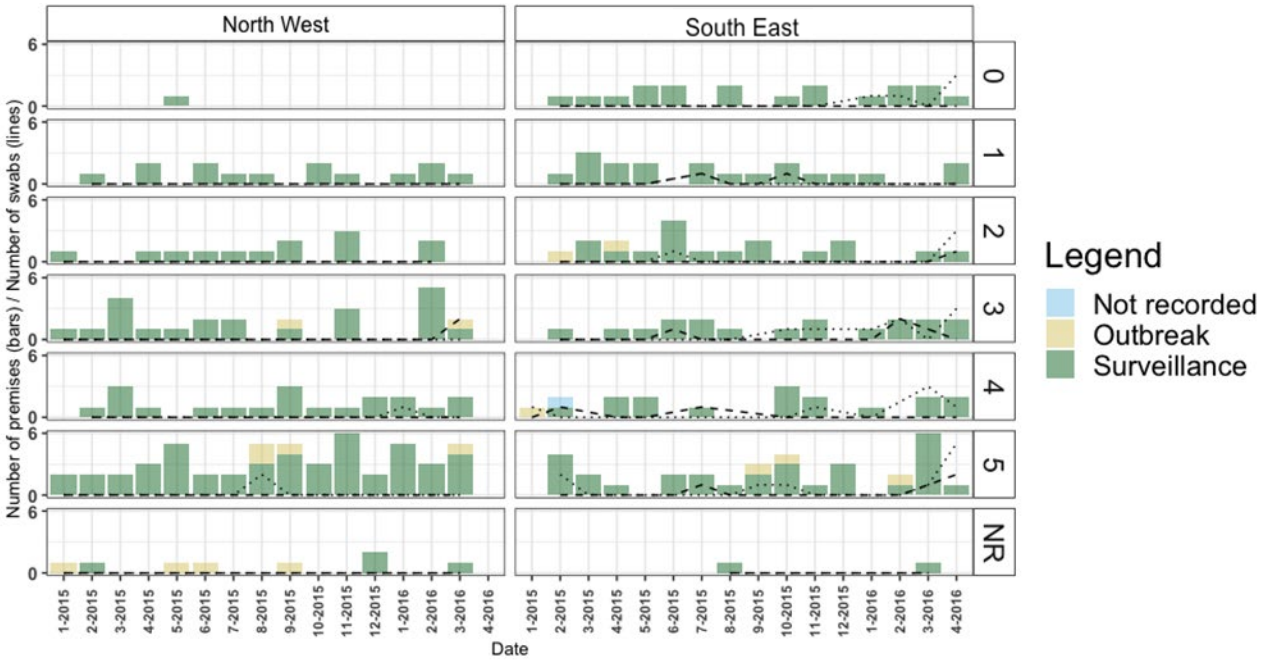

Table 1

Characteristic	Number of premises			Number of samples		
	SE England	NW England	TOTAL	SE England	NW England	TOTAL
General study parameters						
Number of eligible premises	124	132	**256**	-	-	-
Premises sampled for surveillance	118	122	**240**	-	-	-
Premises sampled for outbreak investigation	6	10	**16**	-	-	-
Norovirus results						
Number of virology swabs referred	-	-	-	1,088	950	**2,038**
Surface swabs sampled	-	-	-	831	698	**1,529**
Hand swabs sampled	-	-	-	257	252	**509**
Norovirus positive results	27	3	**30**	45	5	**50**
Norovirus undetermined samples (inhibitory)	-	-	-	19	16	**35**
Positive Surface Swabs	-	-	-	32	3	**35**
Positive hand swabs	-	-	-	13	2	**15**
Bacteriology results						
Number of bacteriological indicator swabs referred	106	121	**227**	411	533	**944**
Number of unsatisfactory bacteriology swab results	71	95	**120**	106	94	**200**
Number of samples with an unsatisfactory ACC result	-	-	-	6	12	**18**
Number of samples with an unsatisfactory coagulase positive *Staphylococci* result	-	-	-	2	8	**10**
Number of samples with an unsatisfactory *E. coli* result	-	-	-	7	7	**14**
Number of samples with an unsatisfactory Enterobacteriaceae result	-	-	-	102	84	**186**
Number of premises with two or more bacteriological test results being reported as unsatisfactory	10	16	**26**	-	-	-