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An outbreak of viral gastroenteritis associated with consumption of sandwiches: implications for the control of transmission by food handlers

U. D. PARASHAR[1,2]*, L. DOW[3], R. L. FANKHAUSER[2], C. D. HUMPHREY[4], J. MILLER[3], T. ANDO[2], K. S. WILLIAMS[5], C. R. EDDY[3], J. S. NOEL[2], T. INGRAM[3], J. S. BRESEE[2], S. S. MONROE[2] AND R. I. GLASS[2]

[1] *Epidemic Intelligence Service, Epidemiology Program Office, Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA*
[2] *Viral Gastroenteritis Section, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases (NCID), CDC, Atlanta, GA 30333, USA*
[3] *Hamilton County General Health District, Cincinnati, OH, USA*
[4] *Infectious Diseases Pathology Unit, NCID, CDC, Atlanta, GA, USA*
[5] *Family Medicine Program, Brown University School of Medicine, Providence, RI, USA*

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SUMMARY

Although food handlers are often implicated as the source of infection in outbreaks of food-borne viral gastroenteritis, little is known about the timing of infectivity in relation to illness. We investigated a gastroenteritis outbreak among employees of a manufacturing company and found an association (RR = 14·1, 95% CI = 2·0–97·3) between disease and eating sandwiches prepared by 6 food handlers, 1 of whom reported gastroenteritis which had subsided 4 days earlier. Norwalk-like viruses were detected by electron microscopy or reverse transcriptase-polymerase chain reaction (RT-PCR) in stool specimens from several company employees, the sick food handler whose specimen was obtained 10 days after resolution of illness, and an asymptomatic food handler. All RT-PCR product sequences were identical, suggesting a common source of infection. These data support observations from recent volunteer studies that current recommendations to exclude food handlers from work for 48–72 h after recovery from illness may not always prevent transmission of Norwalk-like viruses because virus can be shed up to 10 days after illness or while exhibiting no symptoms.

INTRODUCTION

Food-borne gastroenteritis caused by Norwalk-like viruses is a major public health concern [1, 2]. Large outbreaks caused by these viruses have been associated with the consumption of contaminated shellfish, such as oysters and clams, harvested from sewage-polluted waters [3, 4]. Although infected food handlers have been implicated repeatedly as the source of infection in several outbreaks [5–7], their role in the contamination of foods is less well defined. In particular, important public health questions, such as the duration of infectivity in relation to illness and the role of asymptomatic food handlers in disease transmission have not been thoroughly evaluated and remain a focus of interest, particularly because of the implications for disease prevention.

Exclusion of sick food handlers from work for 48–72 h after cessation of diarrhoea and vomiting has been considered adequate to prevent and control Norwalk-like virus outbreaks related to food handling [2, 5–9]. This recommendation was based on epidemiologic observations made in outbreak investigations and the findings of an early volunteer study which indicated that viral shedding in faecal specimens

* Author for correspondence.

was undetectable by immune electron microscopy (IEM) beyond 100 h after the time of inoculation [10]. However, a recent volunteer study that examined viral excretion in stool by more sensitive enzyme immunoassays (EIA) found that shedding persisted in some individuals for up to 2 weeks after recovery from illness [11] and was also detected in individuals with asymptomatic infections. At present, it is not clear whether the excretion of virus in the absence of symptoms necessarily means a person is infectious, but control measures certainly need to be kept under review as further reports are collected.

On 3 March, 1997, the General Health District in Hamilton County, Ohio, USA, was notified that at least 50 of 325 employees of a company had developed acute gastroenteritis during the preceding 4 days. Apart from environmental exposures at work, the only common event identified was a catered lunch held in the company lunch room on 27 February. No bacterial or protozoal pathogen was identified in faecal specimens from case-patients. On 4 March, we began an investigation to determine the aetiologic agent, source of infection, mode of spread in order to assess the possibility of ongoing transmission, and to recommend control measures.

METHODS

Epidemiologic investigation

On 5 March, we surveyed all company employees to ascertain the symptoms, time of onset, and duration of illness, items of food and drink consumed at the catered luncheon held on 27 February, and illness among family members. A case was defined by the presence of vomiting or diarrhoea ($\geqslant 3$ loose stools in 24 h). We interviewed the food handlers from the local restaurant which supplied food items served at the lunch to obtain information on sources of raw food, mode of preparation and storage of food items, and any absenteeism or symptoms of illness. Information was entered into an Epi-Info (Version 6.0) data file and analysed by using the same software [12]. We compared food-specific attack rates of gastroenteritis by a χ^2 test of significance and calculated rate ratios (RR) with 95% confidence intervals (CI) [13].

Environmental investigations

We inspected the water and sewage systems supplying the company lunch room and obtained available specimens of food, ice, and water served at the lunch to test for bacterial agents. We reviewed kitchen facilities at the local restaurant which catered the lunch and observed the procedures used in the preparation of food items. In addition, we obtained information on the vendors who supplied ingredients used to prepare foods served at the lunch and contacted other establishments supplied by the same vendors to inquire about cases of gastroenteritis among their patrons.

Laboratory investigations

Rectal swabs from 30 sick employees were tested for the presence of *Salmonella*, *Shigella*, and *Campylobacter* species at the laboratory of the Cincinnati Health Department in Ohio. Stool specimens from 16 sick employees and 4 food handlers (1 of whom reported symptoms of gastroenteritis prior to the outbreak) were held at 4 °C and transported to the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, to test for the presence of viral pathogens.

Faecal samples were examined initially by direct electron microscopy (EM) using standard methods [14]. We tested paired sera from three sick company employees and 2 food handlers (including the food handler who reported gastroenteritis prior to the outbreak) for seroconversion to the virus responsible for the outbreak by immune electron microscopy (IEM) using a pooled suspension of three stools positive for Norwalk-like viruses by both direct EM and reverse transcription-polymerase chain reaction (RT-PCR). Anti-human IgG conjugated to colloidal gold was used to identify immunoglobulin complexes, which appeared as viral particles covered with the small black grains ascribed to the conjugated gold [15]. We graded the reaction score from none (0) to moderate ($+2$) on the basis of the density of the grain in this study. A positive IEM response was defined as a convalescent-phase serum reaction which was $\geqslant +1$ score higher than that of the paired acute-phase serum.

Viral RNA was extracted from stool specimens and amplified by RT-PCR using 2 primer sets, G-1 and G-2, which amplify a 123 base region of the RNA polymerase gene of genogroup I and genogroup II viruses, respectively [16]. An additional RT-PCR using mon381 (5′-caa gaa tgt aca atg gtt atg c-3′) and mon382 (5′-tga tag aaa tta ttc cta aca tca gg-3′) as the positive and negative sense primers, respectively, was

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conducted to amplify a 223 base region in the capsid gene [17]. The sequence of the RT-PCR products was determined and analysed by standard methods [16, 17].

RESULTS

Epidemiologic investigations

Of the 234 (72%) company employees who returned the completed survey, 85 (36%) reported symptoms that met the case definition. Thirty-six (42%) of the 85 patients reported onset of illness on 28 February, and 39 (46%) reported onset on 1 March (Fig. 1). Two of the 3 employees whose illness began on the day of the lunch (27 February) had onset of illness at 6 p.m. while the third had onset of illness at midnight. The symptoms most commonly reported by the patients were nausea (93%), cramps (86%), diarrhoea (71%), and vomiting (70%). The median duration of illness was 18 h (range, 12–48 h), and none of the respondents lost any time from work. One of the 6 food-handlers who prepared sandwiches served at the catered lunch reported a similar illness that had subsided 4 days prior to the event. The other food handlers reported having no illness in either themselves or their family members in the 2-week period prior to or after the outbreak.

Examination of food-specific attack rates of illness indicated that eating sandwiches served at the catered lunch was strongly associated with illness (Table 1). Although 4 different kinds of sandwiches were served, no individual sandwich or sandwich combination could account for all the cases. Of note, 2 employees reported that they had brought sandwiches home from the lunch where they were consumed by two household members who subsequently developed acute gastroenteritis. Although consumption of ice and tap water was also associated with illness in crude analysis, the risk did not persist after stratifying for consumption of sandwiches.

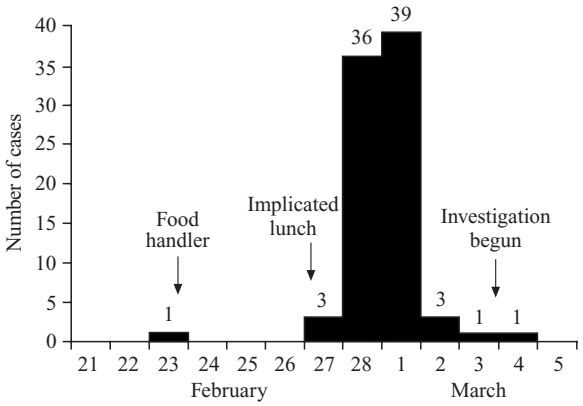

Fig. 1. Gastroenteritis cases by date of onset, Ohio, USA, 1997. Arrows indicate important events during the course of the outbreak investigation including the date of resolution of the food handler's illness, the date when the catered lunch was held, and the date the investigation was initiated.

Environmental investigation

Samples from the water fountain and ice machine at the company contained no evidence of contamination with enteric pathogens. Samples of food showed no bacterial growth. During our review of kitchen procedures, we learned that the sandwiches served at the lunch were prepared at a local restaurant between 7.30 a.m. and 10.30 a.m. on 27 February by 6 food handlers, none of whom routinely used measures such as gloves or suitable utensils to prevent contamination of food. Most ingredients for the sandwiches (e.g., vegetables, meats) were provided by local retailers who supplied other establishments in the vicinity of the implicated restaurant as well. None of the other establishments reported an increase in the number of reported cases of gastroenteritis.

After preparation, the sandwiches were placed on 20 covered platters, refrigerated, and transported to the company at 11.00 a.m. Two company employees (neither of whom reported gastroenteritis prior to or on the day of the lunch) arranged 12 of the 20 platters on tables in the lunch room without any handling of the contents. The remaining platters were refrigerated for the employees who reported at 4 p.m. for the evening shift. Between 11.30 a.m. and 2 p.m., company employees on the day shift served themselves from the platters. Staff members who arrived for the evening shift served themselves from the platters in the refrigerator. Gastroenteritis was reported by company employees who worked on the day shift as well as those who worked in the evening.

Laboratory investigations

All rectal swab specimens from the sick employees were negative for *Salmonella*, *Shigella*, and *Campylobacter* species. Norwalk-like viruses with amorphous surfaces resembling those of prototype Norwalk virus were identified by direct EM in 9 of 20 stool specimens examined, including the specimen obtained from the sick food handler who was ill 4 days before the lunch

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Table 1. *Food-specific attack rates of gastroenteritis among company employees, Ohio, USA, 1997*

	Persons who consumed the item		Persons who did not consume the item		
Food item	Number with illness/ total number	Attack rate (%)	Number with illness/ total number	Attack rate (%)	Relative risk (95% CI)
Sandwiches	82/199	41	1/34	3	14·1 (2·0–97·3)
Ice	63/157	40	20/75	27	1·5 (1·0–2·3)
Tap water	21/42	50	62/191	33	1·5 (1·1–2·2)
Chips	60/158	38	20/75	27	1·4 (0·9–2·1)
Cookies	63/162	39	20/71	28	1·4 (0·9–2·1)
Bottled soda	55/139	40	28/94	30	1·3 (0·9–1·9)
Canned soda	15/33	46	68/200	34	1·3 (0·8–2·0)

Table 2. *Laboratory results from clinical specimens from company employees and food handlers with gastroenteritis, Ohio, USA, 1997*

Status, patient number	Direct electron microscopy	Polymerase chain reaction*
Company employee		
1	Positive	Negative
2	Negative	Negative
3	Negative	Negative
4	Positive	Positive
5	Negative	Negative
6	Negative	Negative
7	Positive	Positive
8	Negative	Negative
9	Negative	Negative
10	Positive	Positive
11	Negative	Negative
12	Positive	Positive
13	Positive	Positive
14	Negative	Negative
15	Negative	Positive
16	Positive	Negative
Food handler		
17	Positive	Negative
18	Positive	Positive
19	Negative	Negative
20	Negative	Negative

* Performed with primers specific for genogroup II Norwalk-like viruses.

(Table 2). The sick food handler's stool specimen was obtained 6 days after the lunch or a total of 10 days after recovery from illness. A positive immune response was determined by IEM for sera from 3 of 3 employees tested and the sick food handler.

Norwalk-like viruses were detected by RT-PCR using primer set G-2, but not G-1, in 7 of 20 faecal specimens, including 6 from company employees and 1 from an asymptomatic food handler whose sera did not demonstrate a positive immune response to IEM. The food handler who was positive by RT-PCR but was asymptomatic was the sister of the sick food handler and did not suffer gastroenteritis either before or after the outbreak. However, the specimen from the sick food handler in which we identified Norwalk-like viruses by direct EM and IEM tested negative by RT-PCR. Because this negative result might have been caused by inhibitor(s) of the RT-PCR reaction, we spiked the sick food handler's specimen with a genogroup I strain of Norwalk-like virus and amplified the mixture using G-1 primers. We were unable to obtain any amplification products in the spiking experiment and, therefore, confirmed inhibition of the RT-PCR reaction.

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The sequences of an 81-base region of the RNA polymerase gene of the RT-PCR products obtained from stool of the asymptomatic food handler and 5 company employees were identical, and showed 93·8% similarity with that of Melksham virus (GenBank accession number: X81879), which is a genogroup II Norwalk-like virus. Because Melksham virus has been genetically classified in the Snow Mountain virus cluster on the basis of a capsid protein [17], we later amplified a 223-base region in the capsid protein using the stool sample from the asymptomatic food handler as the source of the template RNA. The phylogenetic analysis of a 175-bp sequence, excluding the 2 primer regions, showed a 95·5% nucleotide and 100% amino acid similarity with Snow Mountain virus (GenBank: L23831, L75682).

DISCUSSION

The clinical characteristics of illness (short incubation period, short duration high rate of nausea and vomiting, absence of fever of dysentery) in this outbreak were consistent with a Norwalk-like virus aetiology that was confirmed by laboratory testing [1, 2]. The epidemiological evidence strongly implicated sandwiches served at the catered lunch on 27 February as the main vehicle of infection. We detected Norwalk-like viruses in stool specimens from 2 of the 6 food handlers who prepared the sandwiches, 1 of whom reported gastroenteritis 4 days prior to the lunch but was asymptomatic when the sandwiches were prepared while the other reported being well throughout the 2-week period before and after the lunch. The virus strains from the asymptomatic food handler and 5 company employees were identical in an 81-base region of the RAN polymerase gene and paired sera from the sick food handler demonstrated an immune response to the virus present in the employees's stool, strongly suggesting a common source of infection. These findings indicated that either the ingredients (e.g., meats, vegetables) supplied to the restaurant were contaminated and the food handlers and company employees became infected after consuming them, or that the infected food handler(s) contaminated the sandwiches during their preparation.

Because groups at other facilities who ate sandwiches prepared with ingredients obtained from the same supplier as that of the implicated restaurant did not develop gastroenteritis, it is likely that the food handlers contaminated the sandwiches during their preparation, particularly since they did not routinely wear gloves when handling ingredients used to prepare the sandwiches. Unfortunately, the presence of RT-PCR inhibitors precluded the establishment of a molecular link between the viral strains in the stool specimen of the sick food handler and company employees. However, the demonstration of a common viral strain in the stool specimens from the asymptomatic food handler and 5 company employees and an immune response to the virus obtained from company employees in paired sera from the sick food handler strongly suggests a common source of infection and links the company outbreak to the sandwich facility. While we cannot be certain, the fact that the 2 food handlers who were excreting virus were sisters who often visited each other at home further supports the possibility that the same virus strain may have been transmitted from 1 to the other through close personal contact and either one could have contaminated the sandwiches.

During this investigation, we considered alternative hypotheses and exposures that could have caused the outbreak. Because faecal bacteria were not found in samples from the ice machine and water fountains in the lunch room, these exposures were not considered likely to have been the source of infection for the outbreak. Person-to-person transmission of virus, as well as spread through aerosolized vomit particles, appeared unlikely because all company employees reported being well prior to and during the lunch and the three employees who became ill on the day of the lunch had their first symptom during the evening after the meal had been served. For the same reason, and because company employees on the evening shift became sick after eating sandwiches from platters that were stored in the refrigerator, it appeared unlikely that the sandwiches were contaminated in the lunch room.

The detection of viral excretion 10 days after recovery from illness and in the absence of clinical symptoms in this investigation is consistent with shedding patterns observed in recent volunteer studies [10], and challenges recommendations concerning the practice of exclusion of sick food handlers from work and ensuring their cleanliness in preparation of food. Although virus shedding is greatest during acute illness and the amount of virus excreted decreases rapidly with recovery [18], this low level of shedding may be important because the infectious dose of Norwalk-like viruses is extremely small (10–100 virions), an even small-inoculum contamination can result in large outbreaks. The exclusion of infectious

food handlers from work for prolonged periods is theoretically possible but is hard to implement, and is not feasible for infected food handlers who are asymptomatic. Although hand washing is recommended, food handlers have no standards similar to the pre-operative scrub recommended for surgeons. Virus is known to persist under fingernails [19], and Norwalk-like viruses are known to resist chlorine disinfection suitable to kill most bacteria [20]. In the absence of more information on the behaviour of viruses in hand washing solutions, a general recommendation for cleanliness is unlikely to interrupt outbreaks such as the one described here.

Specific control measures are needed to prevent outbreaks of gastroenteritis due to food handlers. These include using of suitable utensils such as deli tissue, spatulas, tongs, single-use gloves or dispensing equipment while handling foods to reduce the chances of contamination, educating food handlers regarding the need for and techniques of adequate handwashing, and regularly inspecting food establishments using the Hazard Analysis and Critical Control Point (HACCP) concepts [21]. Cold, ready-to-eat foods are implicated most often as the source of infection in outbreaks of food borne viral gastroenteritis [2], and individuals handling these foods should be subject to the highest standards of hygiene, both personal and in food handling practices. Future investigations of outbreaks of food-borne viral gastroenteritis should specifically address issues such as the duration of shedding after illness, infectivity of food handlers who shed virus without exhibiting symptoms of gastroenteritis, and the role of sub-optimal public health practices such as the failure to adequately wash hands or to avoid bare-hand contact of ready-to-eat foods so that recommendations for the control of these common outbreaks can be improved.

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