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SHORT REPORT
Foodborne outbreak of gastroenteritis due to Norovirus and *Vibrio parahaemolyticus*

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SUMMARY

Vibrio parahaemolyticus and Norovirus have been recognized as the cause of sporadic cases or outbreaks of diarrhoeal illness in association with the ingestion of raw or improperly cooked seafood. This report describes a foodborne outbreak of gastroenteritis caused by both Norovirus and *Vibrio parahaemolyticus* following the consumption of raw seafood in a restaurant in Terrassa (Catalonia, Spain) in September 2005. Measures are needed to reduce contamination of raw seafood. Consumers can reduce the risk of foodborne illness by avoiding consumption of raw or undercooked food.

Key words: Foodborne outbreak, Norovirus, *Vibrio parahaemolyticus*.

Norovirus (NoV) is a leading cause of outbreaks of foodborne gastroenteritis but outbreaks due to *Vibrio parahaemolyticus* (Vph) are uncommon. Outbreaks with more than one microorganism detected are even more unusual. There are some reports of waterborne [1] or foodborne outbreaks [2, 3] where NoV and bacteria have been found in stools of one or more patients. Other reports [4, 5] have found NoV in some patients and different pathogens in other patients involved in the same gastroenteritis outbreak, but the precise role of each microorganism could not be determined.

After a 12–48 h incubation period, the main symptoms of NoV infection are nausea, vomiting and diarrhoea, and low-grade fever lasting 1–3 days. Symptoms are usually mild and complications are rare. Subclinical infections also occur in some individuals. Noroviruses are transmitted through the faecal–oral route, due to consumption of faecally contaminated food or water, direct person-to-person spread or environmental and fomite contamination [6]. Contaminated oysters and other bivalve molluscs have often been identified as the source of NoV gastroenteritis [7–9]. Elimination of this risk during cooking requires a temperature of 90 °C in the interior of the mollusc for at least 1·5 min [10].

Vph is distributed worldwide in tropical and temperate marine coastal and estuarine waters. Most infections occur during the summer, when warmer water temperatures favour the growth of the organism. Diarrhoeal illness is the most common form of disease caused by Vph and usually occurs 4–96 h after

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ingestion of raw or improperly cooked seafood. Clinical manifestations include diarrhoea, potentially severe abdominal cramps, nausea, and fever that rarely exceeds 38·9 °C [11]. Although infrequent, severe diarrhoea and dysentery-like illness with blood and mucus in the stool have been described. Most cases of Vph gastroenteritis are self-limiting with a mean duration of 3 days. Heating at 60 °C for 15 min kills Vph and storage at $\leqslant$4 °C inhibits its growth [12].

On 8 September 2005, an outbreak of acute gastroenteritis affecting three people was reported to the Epidemiological Surveillance Unit of the Central Region of Catalonia (Spain) by a general practitioner. Epidemiological investigation began as soon as the outbreak was notified. Information was obtained on the clinical symptoms, onset of illness and food items.

The three patients, two women and a man, all aged 40–50 years, had dinner in a seafood restaurant in Terrassa (Catalonia, Spain) on 4 September 2005, together with another person who did not become ill. The mean onset of the common symptoms of the three patients was 16 h (19, 17, and 14 h respectively). Symptoms included watery profuse diarrhoea, severe abdominal pain, nausea and vomiting, and headache. Two patients had a temperature of 39 °C, and one suffered severe myalgia and bloody diarrhoea. The diarrhoea did not remit completely for a mean of 10 days. Patient no. 1 was a 47-year-old woman, who suffered one episode of bloody diarrhoea, severe abdominal pain and profuse diarrhoea lasting for 1 week, stools were positive for NoV and culture negative for Vph but samples were taken at day 4 of symptoms. Patient no. 2 was a 43-year-old man, the husband of patient no. 1, the last episode of diarrhoea was 10 days after disease onset, but abdominal pain persisted for several more days, Vph stool culture and detection of NoV were positive. Patient 3 was a 54-year-old woman with very profuse diarrhoea and vomiting, recovery was not complete after $>$2 weeks, stools were not analysed.

All patients had consumed an assorted shellfish dish which included raw and steamed bivalve molluscs. The person who did not become ill did not consume raw or steamed seafood, he ate only different types of fried fish. Nobody ate the lettuce present on the plate of seafood. Active investigation revealed no associated illnesses in other health centres in Terrassa.

Stools of two patients and four food handlers were analysed for bacteria and viruses at the Microbiology Service of Vall d'Hebron Hospital (the reference laboratory for foodborne outbreak investigations in Catalonia). Stool samples were plated on selective and differential media to study *Salmonella* (MacConkey agar, *Salmonella–Shigella* agar, xylose-lysine-desoxycholate agar and selenite enrichment broth), *Shigella* (MacConkey agar and *Salmonella–Shigella* agar), Shiga toxin-producing strains of O157:H7 *Escherichia coli* (MacConkey agar with sorbitol), *Yersinia* (cefsulodin–irgasan–novobiocin, CIN agar), *Campylobacter* (charcoal agar), *Vibrio* (thiosulfate citrate bile salt sucrose, TCBS agar) and *Aeromonas* spp. (*Pseudomonas–Aeromonas* agar with 100 000 IU/litre of penicillin G, GSP agar). The specific virulence genes from *Escherichia coli* enterohaemorrhagic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC) and enterotoxigenic (ETEC) samples were characterized by conventional PCR with specific primers. Enteric pathogen strains were identified biochemically by means of the automated identification system (Vitek 2; bioMérieux, Marcy l'Etoile, France). The antibiotic susceptibility of enteric pathogen strains was determined by the disk diffusion method on Mueller–Hinton agar plates using Rosco disks (Neo-Sensitab; Rosco Diagnostica, Tastrup, Denmark). Reverse transcriptase–polymerase chain reaction (RT–PCR) detection was performed for NoV detection. The primers used for NoV RT–PCR were NVp110 (5′-ACD ATY TCA TCA TCA CCA TA-3′) for RT and JV12 (5′-ATA CCA CTA TGA TGC AGA TTA-3′) and JV13 (5′-TCA TCA TCA CCA TGA AAA GAG-3′) for PCR. In the reaction two controls, one positive and another negative (sterile water), were always included.

Patient no. 1 was positive for NoV and patient no. 2, was positive for NoV and Vph. Food handlers did not have symptoms for 2 weeks before or after the outbreak, but one was positive for NoV. It was not possible to identify the genogroup nor the sequence of the NoV detected and it was referred by the laboratory as non-typable.

Food handlers were asked about food preparation and handling. The food handler positive for NoV had cleaned all the seafood and helped in the preparation of assorted shellfish dishes, which were prepared several hours before being served. Spider crabs, gooseneck barnacles and blue crabs were boiled for about 17 min in the morning, around 10 h before being consumed, and may have remained at room temperature for more than 1 h to cool. The clams and oysters were cleaned and opened just before serving

and were served raw. The shellfish were served on a bed of lettuce strips, which were cut during the morning and washed without disinfectant in the same sink that the seafood was cleaned in. No samples of the consumed food remained for analysis.

Inspection of the kitchen and the refrigeration chambers revealed many deficiencies that could have facilitated cross contamination of foods, including dirt and food remains in corners, the use of cloth rags to wipe surfaces and hands, non-manual taps and handwashing facilities used for other purposes. Dead bivalve molluscs were on display under glass as fresh products.

The outbreak and inspection of the restaurant's facilities led the health authorities to close the restaurant as a precautionary measure.

Gastroenteritis outbreaks produced by two different microorganisms are infrequent. However, as bivalve molluscs are reservoirs of both NoV and Vph, similar outbreaks may be more frequent than is supposed. In this mixed outbreak, other factors, such as incorrect food handling, inadequate refrigeration, insufficient boiling, cross contamination and recontamination may have been involved, since some of the seafood served was handled after cooking.

Vph outbreaks are rare in Spain. Clinical suspicion in individual patients is low and microbiological studies are often not performed except when a foodborne outbreak is investigated by an Epidemiological Surveillance Unit. Then most microbiology laboratories have to be notified that Vph illness is suspected and the appropriate methods to isolate the organism must be used. Therefore, Vph outbreaks and, even more frequently, mixed outbreaks may be underdetected. Consumption of raw or undercooked seafood during the 48 h before the onset of gastroenteritis symptoms, even in individual cases, should be sufficient reason for performing microbiological studies to detect Vph. The symptoms and incubation period of Vph and NoV may be similar, but when patients present severe symptoms, such as fever and bloody diarrhoea, Vph infection needs to be ruled out, especially if shellfish consumption is suspected. In the outbreak described, despite the differing microbiological results of the three cases, probably all of them were infected by NoV and Vph. In fact the combined infection of Vph and NoV could be the reason for the more severe symptoms and the more prolonged duration of the disease in the cases detected.

As the natural habitat of Vph is marine coastal environs, it is impossible to avoid contamination of seafood by this organism. Hygienic measures, refrigeration or freezing to avoid multiplication and cross contamination between cooked food and raw seafood, and adequate cooking may be the best preventive measures against Vph infection [12].

Given the potential for outbreaks associated with cultivated bivalve molluscs and the possibility of adopting control measures which protect consumers [13], mandatory reporting of Vph infection should be considered and Vph isolates should be referred to public health laboratories to confirm strain subtyping, at least during outbreaks.

The highly infectious potential of NoV must also be considered, since 10–100 particles can be sufficient to cause infection. Similarly, NoV is resistant to refrigeration, heating at 60 °C for 30 min, chlorine concentrations of 0.5–1 mg/litre, and to detergents [14]. Moreover, depuration of seafood is insufficient to eliminate NoV completely [15].

Both Vph and NoV have emerged in recent years as the cause of sporadic cases and epidemic outbreaks of gastroenteritis worldwide, whose full role in human health and disease has yet to be determined [16]. Effective prevention strategies that take into account the complete food chain from distribution to kitchen, including shellfish harvesting areas, are required. Consumers should be made aware of the increased risk of infection associated with eating raw seafood.

Measures are needed to reduce contamination of raw seafood and consumers can reduce the risk of foodborne illness caused by NoV and Vph by avoiding consumption of raw or undercooked molluscs.

Testing for a range of organisms should be considered to determine the cause of foodborne outbreaks as mixed viral-bacterial origins of gastroenteritis cases and outbreaks are more frequent than currently supposed [3, 17].

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DECLARATION OF INTEREST

None.

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