A FOODBORNE NOROVIRUS OUTBREAK AMONG THE FILM CREW IN THE AUTONOMOUS PROVINCE OF VOJVODINA, SERBIA

EPIDEMIJA TROVANJA HRANOM IZAZVANA NOROVIRUSOM MEĐU ČLANOVIMA FILMSKE EKIPE U AUTONOMNOJ POKRAJINI VOJVODINI, SRBIJA

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Summary

Introduction. Norovirus is one of the most common causes of acute gastroenteritis in the world. The aim of this study was to describe characteristics of an outbreak of norovirus infection and present all the steps of an outbreak investigation. Material and Methods. A retrospective cohort study was conducted among all individuals who ate food from the same restaurant. All the exposed persons filled out an adapted questionnaire that is routinely used in patients with food poisoning. Results. This paper presents a norovirus outbreak among film crew members and measures taken to control the epidemic. Conclusions. Although the source of this outbreak remains unclear, the results of environmental investigation of the restaurant showed several irregularities in the kitchen that may have caused norovirus transmission. Key words: Norovirus; Disease Outbreaks; Foodborne Diseases; Gastroenteritis; Surveys and Questionnaires; Epidemiology; Signs and Symptoms; Risk Factors

Acknowledgement

We would like to respectfully acknowledge the following colleagues who participated in the investigation and control of this outbreak: Vesna Milošević and Aleksandra Patić (Center of Virology, Institute of Public Health of Vojvodina, Novi Sad, Serbia) and Biljana Radosavljević (Center of Microbiology, Institute of Public Health of Vojvodina, Novi Sad, Serbia) for analysis of stool samples; Vera Guzman and Goran Pavlović (Center of Microbiology, Institute of Public Health of Vojvodina, Novi Sad, Serbia) for analysis of food, nosophygeal swabs and environmental samples. Special thanks go to Dr. Predrag Đurić and Prof. Gorana Dragovac, for their helpful suggestions on the article.

Introduction

Norovirus (NoV), a member of the Caliciviridae family, is a single-stranded ribonucleic acid (RNA) virus with low infectious dose. It is one of the most common causes of acute gastroenteritis in the world and affects all age groups, with the most common genogroup II, genotype 4 [1, 2]. Symptomatic NoV infection is characterized by acute onset of nausea, vomiting, abdominal cramps, and diarrhea [3]. In foodborne outbreaks caused by NoV, the food can be contaminated by an infected person who is in the acute phase of the disease, but also by food handlers who are asymptomatic carriers [2]. Viruses, including NoV, are difficult to isolate from food, in contrast to bacteria, which can replicate in food. Therefore, identification of viral pathogens in the stool of patients and comprehensive

Sažetak

Uvod. Norovirus je jedan od najčešćih uzročnika akutnog gastroenteritisa u svetu. Cilj ovog istraživanja bio je da se opišu karakteristike jedne epidemije izazvane norovirusom i prikažu sve etape u sprovođenju istraživanja epidemije. Materijal i metode. Sprovedena je retrospektivna kohortna studija među svim osobama koje su konzumirale hranu iz istog restorana. U istraživanju je za sve eksponirane osobe korišćen upitnik prilagođen za anketiranje pacijenata kod trovanja hranom. Rezultati. Ovaj rad prikazuje epidemiju norovirusom među članovima filmske ekipa i preduzete mere kontrole ove epidemije. Kod ukupno 20 izloženih osoba registrovan je gastroenteritis. Kod većine pacijenata, simptomi su trajali 1−3 dana, a svaki treći pacijent zatražio je medicinsku pomoć. U kliničkoj slici dominirali su povraćanje i proliv. Inkriminisana namirnica bio je sendvič sa šunkom, sirom i zelenom salatom. Mikrobiološkim ispitivanjacima je potvrđeno da je uzročnik epidemije norovirus. Zaključak. Iako je izvor ove epidemije ostao nepoznat, rezultatima ispitivanja radne sredine pretpoznato je nekoliko nepravilnosti u kuhinji restorana, čime je verovatno omogućeno širenje norovirusa. Ključne reči: Norovirus; epidemija bolesti; bolesti uzrokovane hranom; gastroenteritis; istraživanja i upitnici; epidemiologija; znaci i simptomi; faktori rizika
epidemiological investigation are very important for the detection of outbreaks caused by NoV [3].

Due to the lack of laboratory diagnostic facilities and limited surveillance of NoV infections in Serbia, there is no data about the incidence in the population, but common reports of gastroenteritis with probable viral etiology [4] indirectly indicate that NoV infection is present in our country with a similar incidence as in the European Union [5].

On the 15th December 2015, a physician from an Emergency Department informed the Center for Disease Control and Prevention of the Institute of Public Health of Vojvodina (IPHV) about five members of a film crew with symptoms of diarrhea and/or vomiting. Those members were admitted on the same morning. Within the next four hours, an additional crew member appeared with similar symptoms. All patients had eaten food from the same restaurant. Immediately after receiving this information, an IPHV Outbreak Team initiated an outbreak investigation, and in cooperation with the sanitary and veterinary inspection, visited the Emergency Department, the restaurant, and the patients’ accommodation.

The aim of this investigation was to find the source and all possible modes of transmission during this outbreak, and to identify and exclude potential risk factors.

**Material and Methods**

A retrospective cohort study was conducted among all subjects who ate food that was delivered from a restaurant to the patients’ accommodation. We defined an outbreak case as a person who ate some meals from the same restaurant for two days (on the 13th and 14th December 2015) and developed diarrhea and/or vomiting within the following three days. Information on the process of preparation, serving and food delivery to the patients’ accommodation during these two days in collaboration with the sanitary inspection were obtained. The investigators adapted a standard questionnaire, routinely used for patients with foodborne diseases. The questionnaire was based on the information from the restaurant with a list of food items which were served during the two days before the outbreak occurrence. Additionally, there were questions on demographic data such as the time and date of onset, clinical features, and the duration of specific signs/symptoms of NoV infection, as well as the time of food consumption.

After data collection, a descriptive and univariate analysis was carried out. Also, the relative risk (RR) values with 95% confidence interval (CI) were calculated. Statistical significance was set at p <

### Table 1. Demographic and clinical characteristics of outbreak cases due to norovirus infection

| Characteristics/Karakteristike | Number of cases/Broj slučajeva* |
|-------------------------------|---------------------------------|
| Sex/Pol                       |                                 |
| Male/Muškarci                 | 11                              |
| Female/Žene                   | 9                               |
| Age (years)/Uzrast (godine)   |                                 |
| 20 - 29                       | 14                              |
| 30 - 39                       | 4                               |
| 40 - 49                       | 2                               |
| Place of residence/Mesto prebivališta |          |
| Bečej                         | 2                               |
| Others **/Ostalo              | 18                              |
| Duration of symptoms//Trajanje simptoma |               |
| Less than one day/Kraće od jednog dana | 2                        |
| 1 - 3 days/Jedan do tri dana  | 12                              |
| ≥ 3 days/Tri i više dana      | 6                               |
| Seeking healthcare/Poseta zdravstvenoj ustanovi | 6                        |
| Hospitalization/Hospitalizacija | 0                               |
| Deaths/Smrtni ishod           | 0                               |

*One member of the film crew reported to have had diarrhea one night before the others, but he did not meet the case definition and was not included in the outbreak cases; **Cases from other places of Serbia (Belgrade, Aleksandrovac, Palić)

**Legenda: *Jedan član filmske ekipe prijavio je da je imao dijareju jednu noć pre ostalih, ali on nije ispunio definiciju slučaja i nije bio uključen kao slučaj ove epidemije; ** Slučajevi iz drugih mesta Srbije (Beograd, Aleksandrovac, Palić)
Results

Epidemiological investigation
None of the interviewed food handlers or kitchen employees reported having been sick in the weeks before to the outbreak, but the chief cook was not at work on the day when the outbreak was investigated.

Descriptive epidemiology
A total of 30 film crew members filled out the questionnaire and 20 of them were diagnosed with infection. Out of total cases, 11 were males and 14 were aged between 20 and 29 (age range of 21 – 44 years). Of all the film crew members, only two patients with symptoms were from the place of outbreak, while the rest were from other places in Serbia. In most patients (12/20) the symptoms lasted 1 – 3 days, and every third patient sought medical attention (Graph 1).

In regard to symptoms, all patients reported vomiting and/or diarrhea. In 80% of all patients, nausea was the symptom with the longest duration (more than three days), but in 95% of all patients, vomiting lasted less than a day. None of them had fever, chills or vomiting longer than three days (Graph 2).

The date and time of symptom onset for 20 cases are shown in Graph 3. In accordance with the information about two different dates of exposure to suspicious food items, most cases had symptoms 40 hours after consuming the food on the 13th December, and 16 hours after food consumption on the 14th December, with the most registered cases on the 15th December, between 02.00 to 04.00 a.m. Analytic epidemiology
There were three meals a day (breakfast, lunch and dinner). All three meals were prepared in the kitchen of the restaurant and delivered to the members of the film crew who were accommodated in their apartments, a few kilometers away from the restaurant. The meals were delivered by a vehicle which was not equipped for safe food transportation.

Considering the two-day period before the outbreak, we identified a total of six food items that were probably related to symptom development. The same food items (sandwiches) were served on both days during breakfast, leading to the highest attack rate among the exposed subjects (RR: 2.8 vs. RR: 3.9, p < 0.05). Although the RR for green beans was more than one, there was not a statistically significant difference among the exposed persons and those who were not (Table 2).

Environmental investigation
After the sanitary inspection, there were several irregularities in the restaurant’s kitchen. More precisely, there were incorrect washing routines, storage of fresh foods and prepared meals, as well as inadequate management and control of cooling the heat-treated foods. In addition, there was evidence of insufficient cleaning and disinfection of work surfaces in the kitchen. Furthermore, there

Graph 1. Frequency of clinical signs/symptoms of outbreak cases due to norovirus infection
Grafikon 1. Učestalost kliničkih znakova/simptoma među obolelima u epidemiji izazvanoj norovirusom

Graph 2. Duration of signs/symptoms of outbreak cases due to norovirus infection
Grafikon 2. Trajanje kliničkih znakova/simptoma među obolelima u epidemiji izazvanoj norovirusom

Graph 3. Epidemic curve of outbreak cases due to norovirus infection by date and time of onset in regard to food consumption
Grafikon 3. Epidemijska kriva - slučajevi epidemije izazvani norovirusom po datumu i vremenu početka tegoba u odnosu na vreme konzumiranja namirnica
were cockroaches on the floor and the walls of the kitchen during the investigation. The kitchen staff did not routinely wear gloves when handling ingredients and food items. In light of the observed irregularities, the sanitary authorities immediately ordered a sanitary examination of food handlers and all the kitchen staff. On the 15th December, after the sanitary inspection, the restaurant’s kitchen was closed, until the correction of all sanitary defects.

In accordance with the Guidelines for microbiological criteria in Serbia [6], samples obtained from the kitchen work surfaces and the cutlery, showed a large number of Coliform bacteria, which is a common bacterial indicator of sanitary quality of food and water.

**Microbiological investigation**

The stool samples of two patients and 11 members of the kitchen staff were tested for bacterial pathogens (Salmonella spp., Shigella spp., Campylobacter spp.) and viruses (norovirus, rotavirus and astrovirus) at the microbiology and virology laboratories of the IPHV. The stool samples were extracted using the Ribo Virus commercial kit.

| Date       | Meal       | Food                              | Number of outbreak cases | Total number of participants/ Ukupan broj ispitanika | Attack rate Učešće oboljelih u oboljelih u epidemiji (%) | Number of cases | Total number of participants/ Ukupan broj ispitanika | Attack rate Učešće oboljelih u ukupnom broju neizloženih (%) | p          |
|------------|------------|-----------------------------------|--------------------------|------------------------------------------------------|-----------------------------------------------------|----------------|------------------------------------------------------|---------------------------------------------------------------|-----------|
| 13. 12. 2015| Breakfast  | Sandwiches with ham, cheese and lettuce salad/ Sendviči sa šunkom, sirom i zelenom salatom | 17                        | 20                                                    | 85.0                                                 | 3              | 10                                                    | 30.0                                                           | 2.8       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (1.1-7.4) |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | 0.0343   |
|            | Lunch      | Moussaka (mashed potatoes, eggs and minced meat)/ Musaka (krompir, jaja i mleveno meso) | 13                        | 22                                                    | 59.1                                                 | 7              | 8                                                     | 87.5                                                           | 0.7       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (0.4-1.0) |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | 0.0771   |
|            | Dinner     | Mashed potatoes, roast pork and canned pepper salad/ Pire krompir, svinjsko peceno meso i salata od konzervirane paprike | 15                        | 23                                                    | 65.2                                                 | 5              | 7                                                     | 71.4                                                           | 0.9       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (0.5-1.6) |
| 14. 12. 2015| Breakfast  | Sandwiches with ham, cheese and cabbage salad/ Sendviči sa šunkom, sirom i kupus salatom | 18                        | 21                                                    | 85.7                                                 | 2              | 9                                                     | 22.2                                                           | 3.9       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (1.1-13.3)|
|            | Lunch      | Green beans, bread and canned pepper salad/ Zeleni pasulj, hleb i salata od konzervirane paprike | 16                        | 22                                                    | 72.7                                                 | 4              | 8                                                     | 50.0                                                           | 1.5       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (0.7-3.0) |
|            | Dinner     | Mixed meat (steak, chicken, bacon, sausage), bread and green salad/ Mešano meso (biftek, piletina, slanina, kobasica), hleb i zelena salata | 15                        | 22                                                    | 68.2                                                 | 5              | 8                                                     | 62.5                                                           | 1.1       |
|            |            |                                    |                          |                                                      |                                                     |                |                                                       |                                                             | (0.6-2.0) |

Legend: * Consumed between 8.00 – 10.00 a.m.; ** Consumed between 1.00 – 3.00 p.m.; *** Consumed between 6.00 – 8.00 p.m.
Legendu: * Konzumira se između 8,00 i 10,00 sati; ** Konzumira se između 13,00 i 15,00 sati; *** Konzumira se između 18,00 i 20,00 sati
transmission of the NoV infection to the general populations, without any evidence of secondary cases. Furthermore, it did not have serious public health consequences, as the illness started with nausea, and vomiting lasted less than a day in 95% of all patients. Diarrhea were registered in all cases, and vomiting lasted for two to three days, but the duration can be longer (linked to this outbreak), used the water from the same source and since the investigation of the outbreak did not register an increasing number of cases with gastrointestinal symptoms. Furthermore, according to the recommendations of the Law on Food Safety of the Republic of Serbia [7], the microbiology tests of fresh barbecue sausages showed genus Listeria, species Listeria innocua and a large number of the Enterobacteriaceae. With available laboratory capacities, no further tests were carried out.

Discussion

A sudden onset and short duration of this outbreak where all cases were registered within a 10-hour period, suggested that food was the probable source of the outbreak. Considering the facts that all of the exposed persons, as well as the suspicious restaurant (linked to this outbreak), used the water from the same source and since the investigation of the outbreak did not register an increasing number of cases with gastrointestinal symptoms, we concluded as a potential source of this outbreak. Norovirus was confirmed in the stool samples of two patients (genogroup II), without further genotyping. However, the stool samples of 11 kitchen staff were negative for NoV. Stool samples for bacterial pathogens of two patients and 11 the kitchen employees were negative. Moreover, the nasal and nasopharyngeal swabs of 11 kitchen staff were negative for Staphylococcus aureus and Streptococcus pyogenes.

In accordance with the recommendations of the Sanitary and Veterinary Inspectorates, samples of the kitchen work surfaces, the cutlery and fresh barbecue sausages were tested for bacterial pathogens in the microbiological laboratory of the IPHV. It was not feasible to perform a microbiological analysis of the food items served during the two suspicious days because it was depleted prior to the outbreak investigation.

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customers of the restaurant) had eaten the same food as the outbreak cases, except for the sandwiches. The food handlers have already been identified as a potential source of NoV transmission in several studies [9,12–14]. The identification of asymptomatic food handlers is very important during NoV outbreaks [2,15]. Results of others authors showed that the food handlers could be a source of the outbreak even in a situation when they denied any gastrointestinal illness during the outbreak period. For example, in previously published research, additional investigations showed that the child (infant) of a healthy food handler had been sick with watery diarrhea two days before the food handler prepared the incriminated meals. Hence, this member of the kitchen staff did not need to be sick, but may have been contaminated after changing the baby’s diapers if he did not wash his hands properly [16]. The concentration of NoV excreted in stool samples of infected individuals among the infected people decreases rapidly and becomes practically undetectable within 2–3 days [12]. We were not absolutely sure if asymptomatic kitchen employees had a role in the spread of the virus in this outbreak, because their stool samples were tested more than 72 hours after the first suspicious meal had been served. Interestingly, the kitchen chief of the restaurant was not at work on the day when the outbreak was investigated and he did not provide a stool sample for laboratory testing. In the absence of symptoms among all tested kitchen staff, negative laboratory results of their stool samples, and the absence of appropriate laboratory capacity for testing of paired serum samples from the kitchen employees, we were not able to directly confirm our hypothesis about the source of this outbreak.

On the other hand, several other facts indicated that this outbreak was potentially classified as a foodborne outbreak of NoV infection. The cause of this outbreak was confirmed using RT-PCR method and the clinical features were typical for NoV infection. Despite the fact that the source of infection was not directly confirmed, results of univariate analysis of the exposure showed that eating sandwiches was associated with an increased risk of infection. The sandwiches were previously recognized as the cause of NoV outbreak [17], and lettuce was the common source of infection [18,19]. Furthermore, high levels of Coliform bacteria, which were found on the restaurant’s kitchen work area surfaces, are indicating that the kitchen was unsanitary. Additionally, considering that NoV remains viable on surfaces for as long as two weeks [20] and due to the fact that the food delivery was carried out in lunch boxes by a vehicle not equipped for the safe food transportation, we believe that these facts probably contributed to the contamination of food items before the consumption.

Conclusion

Our report represents the first detailed epidemiological study of a foodborne outbreak due to the norovirus infection in Serbia, and we recognized several gaps during the investigation. For the recognition of the outbreak type, the probable source, the reservoir of infection, as well as the route of transmission, it is necessary to carry out an immediate and comprehensive investigation. Outbreak conclusions and consequently control measures about certain outbreaks may be inadequate without appropriate laboratory support for the confirmation of certain pathogens.

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