Questionnaire-based epidemiological analysis of acute gastroenteritis outbreak among employees of two neighbouring institutions sharing canteen in Prague 2019

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ABSTRACT

Objectives: On 17th Dec 2019 gastroenteritis outbreak occurred in two Prague's neighbouring institutions. Investigation aimed to describe outbreak, identify etiological agent, vehicle and propose control measures.

Methods: Routine outbreak investigation and retrospective cohort study was done. Data collected via online questionnaire were analysed using descriptive, univariate and stratified analysis.

Results: Of 960 employees, 276 responded (29%). We identified 39 (14%) cases, one tested norovirus positive. Canteen staff didn't report illness. No food item or environmental sample was tested. Sichuan pork served for lunch on 17th Dec was the most likely vehicle of outbreak (odds ratio (OR) 5.02, 95% confidence interval (CI) 1.98–12.64). Eating Sichuan pork and Chinese soup showed OR 31.5, 95% CI 5.0–320.7. Twenty-two (56%) cases can be explained by consumption of these food items.

Conclusions: Epidemiological analytical method provided evidence of likely vehicle. We did not find the source. Control measures were early ensured and outbreak ceased. We emphasise full outbreak investigation using analytical epidemiology, environmental screening and microbiological testing of cases and possibly all kitchen staff.

KEYWORDS

outbreak investigation - acute gastroenteritis - cohort study - norovirus - analytical epidemiology

SOUHRN

Liptáková M., Špačková M., Balasegaram S., Orlíková H., Kynčl J.: Epidemiologické šetření a analýza epidemického výskytu akutních gastroenteritid mezi zaměstnanci dvou sousedních institucí sdílejících jídelnu v Praze v roce 2019

Cíle: Dne 17. prosince 2019 došlo ve dvou sousedících pražských institucích k výskytu několika onemocnění akutní gastroenteritidou. Provedené epidemiologické šetření si kladlo za cíl upřesnit rozsah ohniska nákazy, identifikovat etiologické agens, stanovit vehikulum nákazy a navrhnout protiepidemická opatření.

Metody: V ohnisku nákazy bylo provedeno standardní epidemiologické šetření, dotazníkové šetření a následně retrospektivní kohortová studie. Byla provedena deskriptivní analýza dat, univariační a stratifikovaná analýza.

Výsledky: Na dotazník odpovědělo 276 (29 %) z 960 zaměstnanců. Identifikovali jsme 39 případů (14 %), jeden s pozitivním laboratorním výsledkem na noroviry. U zaměstnanců jídelny nemoc nebyla hlášena. Potraviny ani vzorky prostředí nebyly laboratorně testovány. Analýzou bylo zjištěno, že pravděpodobným vehikulem nákazy byla vepřová plec po sečuánsku, podávaná k obědu dne 17. prosince (OR 5,02, 95% Cl 1,98–12,64). Společná konzumace vepřové plece po sečuánsku a čínské polévky vykazovala riziko OR 31,5, 95% Cl 5,0–320,7. Konzumací těchto pokrmů lze vysvětlit 22 případů (56 %).

Závěr: Epidemiologická analýza poskytla důkaz o pravděpodobném vehikulu nákazy. Zdroj nebyl zjištěn. Protiepidemická opatření byla včas nastavena a další onemocnění se nevyskytla. Upozorňujeme na vhodnost využití metod analytické epidemiologie v rámci terénního šetření, provádění environmentálního šetření a mikrobiologického testování případů, případně také všech zaměstnanců kuchyně.

KLÍČOVÁ SLOVA

šetření ohniska nákazy – akutní gastroenteritida – kohortová studie – norovirus – analytická epidemiologie

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PŮVODNÍ PRÁCE

INTRODUCTION

Norovirus, a member of the Caliciviridae family, is described to be predominant cause of acute gastroenteritis worldwide [1], responsible for approximately one fifth of all gastroenteritis cases [2]. Norovirus was the most frequently identified in foodborne outbreaks, the fourth most commonly reported agent detected in foodborne outbreaks in EU; frequently reported in restaurants, pubs, street vendors or take-away food than in any other settings [3]. Common sources include family members, health care workers, contaminated food or water, and the environment [4]. The infection of food handlers may be additional potential source [5]. Different food items have been associated with norovirus outbreaks previously. In principle, any food item may become contaminated if handled by infected person or if washed or humidified with contaminated water [6]. The incubation period of noroviral infection is commonly 1-2 days [7]. The incubation period is frequently used to determine the time of exposure in foodborne outbreaks [8].

On the 19th Dec 2019, the Local Public Health Authority (LHA) in Prague was informed about 12 cases of gastrointestinal illness among employees of the National Institute of Public Health Prague (NIPH) and the State Institute of Drug Control (SIDC). Institutions share common area and the canteen. An outbreak control team (OCT) was established from LHA and NIPH epidemiologists immediately in order to assess the extent of the outbreak, identify potential source and implement control measures to prevent occurrence of additional cases. Initial investigation revealed that the reported symptoms were diarrhoea or nausea (but no fever) during night from 17th Dec evening – 18th Dec 2019. All cases recovered within 24 hours, none sought medical care. All cases declared eating lunch at the canteen on the 17th Dec; most of them ate "Sichuan pork" with jasmine rice or chicken meat with vegetables and potato pancakes. Based on symptoms, their onset and a common denominator, which was eating in the canteen, the OCT hypothesized the point-source outbreak possibly of norovirus origin caused by contaminated food or water in the canteen.

Well-documented foodborne outbreaks due to norovirus are rarely found in the literature. In the Czech Republic (CZ) only very few outbreak investigations (OI) are published internationally. Majority of outbreak reports submitted to Minnistry of Health and the National Institute of Public Health are reports with only descriptive analysis and description of measures applied. Analytical epidemiological methods are very seldom applied in local foodborne outbreaks investigations [9].

We bring forward one of the first comprehensive gastrointestinal OI using epidemiological analytical methods in CZ.

METHODS

Epidemiological investigation

Active case finding within the canteen and employees of both institutions was performed. The definition of the case was determined by self-reported symptoms and by laboratory confirmation. A probable case was defined as employee of the NIPH or SIDC who reported onset of gastrointestinal symptoms (diarrhoea or nausea or abdominal pain or vomiting) from 17th Dec 00:00 hrs. until 19th Dec 24:00 hrs. A confirmed case was defined as probable case with laboratory confirmed norovirus infection.

The OCT hypothesized that the outbreak vehicle would be any item of the menu served on the 17th Dec. The analytical retrospective cohort study design was chosen to assess association of the food items and the outcome. Exposures were defined as the various food and beverage items consumed in the canteen on 16th-18th Dec. The OCT administered an online guestionnaire, which was emailed to all employees of both institutions; to all NIPH email addresses on 19th Dec (n = 510) and SIDC on 20th Dec (n = 450). Data collection was ongoing throughout the Christmas holiday, and it was closed on 8th Jan 2020 as the number of reports remained the same after 9 days follow up. The data were collected through Google Forms. The questionnaire was anonymous, although the respondents with symptoms could voluntarily add their email address. Cases detected by questionnaire were interviewed by phone by LHA staff for more detailed information. As anticipated incubation period is short, specific for the most common gastroenteritis causal agents, we proposed for the epidemic curve construction the time intervals of 8 hrs. The onset of symptoms was categorised as 0-8, 8-16, 16-24 hrs. within the three successive days 17th-19th Dec. The questionnaire included demographic variables (e. g. age and sex), clinical symptoms and specification of food and beverages consumption in the canteen on 16th-18th Dec. Only items included in the menu were included in the questionnaire. We performed descriptive, univariate and stratified analysis using STATA version 16. For assessing the risk associated with different food and beverages we used odds ratio (OR). We set the statistically significant level to 5%; p = 0.05 with 95% confidence intervals (CI). Stratified analysis was performed for exposures that showed strong association with the outcome to identify if effect modification or confounding was present. The X²-test and Wilcoxon-test were used whenever appropriate.

Environmental investigation

LHA staff performed environmental inspection in the canteen using the Hazard Analysis and Critical Control Points method to critically analyse canteen settings, operations and check of food handling practices on 19th Dec. Neither food nor environmental samples were taken for microbiological analysis.

Laboratory investigation

Two symptomatic cases (NIPH employees) and two asymptomatic food handlers were available and provided a clinical stool samples. After medical instructions, the stool samples were taken by patients themselves usually at home. On Friday, 20th Dec 3 stool samples from 2 symptomatic employees and 1 asymptomatic food handler were send to laboratory. One additional sample from other asymptomatic food handler was sent on 27th Dec. No other samples were available. The samples were tested by electron microscopy. No testing for bacterial infectious agents of gastroenteritis was performed.

RESULTS

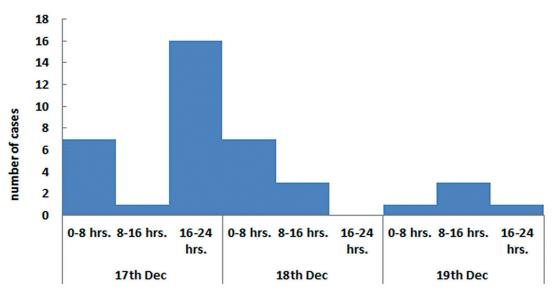
Epidemiological investigation

Of total 960 employees (510 of NIPH and 450 of SIDC), 276 replied to the questionnaire (response rate 28.8%). The demographic characteristics are shown in Table 1. The respondent's age ranged from 24–77 years, mean 47.1, median 49.0. Thirty-nine probable cases were identified (14.1%). The main symptoms were diarrhoea (35 cases, 89.7%), abdominal pain (24 cases, 61.5%), nausea (14 cases, 35.9%) and one case reported vomiting and one case fever (2.6%). The duration of illness ranged from within 12 hrs. (26 cases, 66.7%), 12–24 hrs.

Variable		Number of respondents (n = 276)	Proportion of respondents (%)	Number of cases (n = 39)	Proportion of cases (%)	Proportion of cases among respondents (%)	
Sex	female	208	75.4	29	74.4	13.9	
	male	68	24.6	10	25.6	14.7	
Age group (years)	20–29	36	13.0	4	10.3	11.1	
	30–39	55	19.9	8	20.5	14.5	
	40–49	50	18.1	12	30.8	24.0	
	50–59	90	32.6	10	25.6	11.1	
	60–69	38	13.8	5	12.8	13.1	
	70–79	7	2.5	0	0.0	0	
Employee of	NIPH*	187	67.8	22	56.4	11.8	
	SIDC [#]	15	5.4	3	7.7	20.0	
	unknown	74	26.8	14	35.9	18.9	

Table 1. Demographic characteristics of respondents of gastroenteritis outbreak in Prague, Dec 2019

*the National Institute of Public Health Prague (NIPH) [#]the State Institute of Drug Control (SIDC)



Date and time of symptom onset

Figure 1. Epidemic curve of cases (n = 39) of gastroenteritis outbreak in Prague, Dec 2019

(11 cases, 28.2%) to more than 24 hrs. (2 cases, 5.1%). No one was hospitalised, one case was positive for norovirus (NIPH employee).

The majority of cases occurred on 17^{th} Dec at 16-24 hrs. and on 18^{th} Dec at 0-8 hrs. (Figure 1). Eight cases had onset of gastrointestinal symptoms on 17^{th} Dec at 0-8 hrs., before consuming lunch at the canteen on the same day. One case (the last on the epidemic curve) had onset of symptoms on 19^{th} Dec at 16-24 hrs., which is beyond our expected incubation period for noroviral infection (48 hrs.).

Characteristics of respondents who ate in the canteen on 16th–18th Dec are shown in Table 2. Not all of the 39 cases ate in the canteen each day. No frozen material such as an ice cream or frozen dessert was included in the menu. On the 17th Dec, seventeen of total 32 cases who responded the question ate "Sichuan pork", 15 of 31 cases who responded the question ate "Chinese soup". Twenty-two cases ate either "Sichuan pork" or "Chinese soup" and nine cases consumed both. Overall attack rate was 14.1% (39 cases among 276 exposed to food in the canteen). Attack rates on respective days were not significantly different from each other.

Food items significantly associated with the outcome in univariate analysis are given in Table 3. The highest ORs resulted for "Sichuan pork" (OR = 5.02, 95% CI 1.98– 12.64) and for "Chinese soup" (OR = 2.40, 95% CI 0.97– 5.85). Both we considered for stratified analysis, while "Beef" was further not relevant. The OR 0.13 of "Beef" indicate "protective" effect, meaning that people who ate "Beef" did not eat "Sichuan pork".

Stratified analysis of two statistically significant food items showed, that "Chinese soup" is an effect modifier of the association between "Sichuan pork" and illness comparing the OR of 22.50 (95% CI 3.19–240.61) in cases who ate both and an OR 2.58 (95% CI 0.71–8.94) in cases who only ate "Sichuan pork". "Sichuan pork" is mutually an effect modifier of the association between "Chinese soup" and illness as the OR for those that were exposed to both items is 12.21 (95% CI 1.75–132.44) and those that only ate "Chinese soup" is 1.40 (95% CI 0.37–4.86). However the effect of "Chinese soup" alone is minor compared to "Sichuan pork" thus the main vehicle would likely be "Sichuan pork". The OR for cases who ate "Sichuan pork" and "Chinese soup" together (nine cases and two non-cases) compared to cases who did not eat either of these two foods (nine cases and sixty three non-cases), was 31.50 (95% CI 5.03–320.67).

Every day a one salad was included in the menu. The salad contained a mixture of cooked food and uncooked part. On 16th Dec crab salad with vegetables was served. None of participants reported eating salad on that day in the questionnaire. On 17th Dec mixed salad with pear and chicken nuggets was served. Three participants (non-cases) reported eating this salad. In univariate analysis OR for this salad was 0.00 (Cl 95%, p = 0.354). On 18th Dec chickpea salad with vegetables was served. Three participants (non-cases) reported eating this salad. In univariate analysis OR for this salad was 0.00 (Cl 95%, p = 0.509).

Environmental investigation

Of ten canteen employees, no one reported any health problems themselves or in family members. The canteen offered two soups, six main dishes and three beverages daily. In incriminated days, the kitchen sold

Day of eating in canteen	Number of respondents who ate in canteen (n=276)	Proportion of respondents who ate in canteen (%) Proportion of cases who ate in canteen (n=39)		Proportion of cases who ate in canteen (%)	Proportion of cases eating among respondents eating (attack rate, %)	
16 th Dec	117	42.4	20	51.3	17.1	
17 th Dec	146	52.9	32	82.1	21.9	
18 th Dec	113	40.9	14	35.9	12.4	

Table 2. Exposure (eating in canteen) among respondents of gastroenteritis outbreak in Prague, Dec 2019

Table 3. Food items significantly associated with the outcome (*p*<0.05) of univariate analysis of gastroenteritis outbreak in Prague, Dec 2019

Exposure	Cases			Non-cases			Univariate analysis results		
Food item*	Total	Exposed	% ¹	Total	Exposed	% ²	OR	95% CI	<i>p</i> value
"Sichuan pork"	32	17	53.1	114	21	18.4	5.02	1.98–12.64	0.000
"Chinese soup"	31	15	48.4	114	32	28.1	2.40	0.97–5.85	0.032
"Beef"	32	1	3.1	114	23	20.2	0.13	0.00-0.86	0.021

*altogether 32 food and beverage items consumed in the canteen between 16-18th Dec were tested.

¹Proportion of exposed among all cases

²Proportion of exposed among all non-cases

238 portions of six main dishes and 130 portions of two soups on 16th Dec, 262 portions of six main dishes and total 140 portions of two soups on 17th Dec and 247 portions of six main dishes and 130 portions of two soups on 18th Dec. LHA inspection did not find any shortcomings in the operation of the kitchen and didn't find any violation of procedures.

Laboratory investigation

Two cases of 39 provided stool samples; one was positive for norovirus. The samples from two asymptomatic food handlers were both tested negative.

DISCUSSION

We investigated gastroenteritis outbreak among employees of two neighbouring institutions in Prague, sharing canteen. First cases occurred during the night on 17th Dec 2019, after eating lunch in the canteen on the same day. The OCT expressed suspicion on point source food or water-borne outbreak and retrospective cohort study was performed.

We analysed the food items and beverages eaten in the canteen on the day before and day after the estimated exposure date (17th Dec 2019) and as case we included all 39 probable cases. Response rate to online questionnaire was 28.8% and we consider it was low due to Christmas holiday ongoing. Due to low response rate and many missing values we decided to interpret the data using odds ratios as the measure of association. If participation rate is low, odds ratio offers some protection against non-response bias.

Descriptive analysis showed no difference in proportion of cases among NIPH, SIDC and non-specified employees of either of these institutions. With univariate analysis "Sichuan pork" served with jasmine rice on 17th Dec was identified as the most likely vehicle for this outbreak, (OR 5.02, 95% CI 1.98-12.64). In our case, those exposed to eating either "Sichuan pork" or "Chinese soup" were more likely to develop gastrointestinal symptoms within 2 days after consumption than those who didn't eat this food. "Sichuan pork" explained 16 cases and "Chinese soup" alone explained further 6 cases; these only accounted for 22 of 39 (56.4%) cases in the outbreak. Stratified analysis showed the strongest association when eating both, "Sichuan pork" and "Chinese soup" (OR 31.50, 95% CI 5.03-320.67). These findings support the hypothesis that the vehicle was most possibly a food item served on the 17th Dec. Dose response could not be determined, as we had no such data available.

The observed time between the exposure and symptoms onset among a major proportion of cases that ate in the canteen was consistent with most published estimates for noroviruses incubation period, 1 to 2 days [7].

Noroviruses are relatively resistant in the environment: they can survive freezing as well as high temperatures (up to 60 °C) [6]. No frozen material such as an ice cream or frozen dessert was included in the menu. Every day a one salad, that contained a mixture of cooked and uncooked part was included in the menu. Only non-cases consumed served salads and OR was not statistically significant.

Contamination of the food items or beverages may have occurred by an asymptomatic, norovirus positive food handler or there could be contamination of food or cross-contamination in the canteen. However, eight cases had onset of symptoms already before the above mentioned lunch and another nine cases did not eat the "Sichuan pork" or "Chinese soup". This indicated possible additional airborne spread of the norovirus already before exposure to food. We could not determine if these persons were in direct contact before eating in the canteen. We assume there might be other sources of contamination present; either both foods were contaminated with norovirus (reflecting the higher OR), and thus people that ate both foods have a higher dose of norovirus to cause illness, or there might also be other contaminated food which was not revealed.

We could not confirm that hypothesis further as not all canteen staff was tested. Optionally there might be any other gastrointestinal illness of these cases with these quite common symptoms. There could be also a combination of several factors leading to illness.

The viruses survive long periods on different surfaces [6]. As extensive contamination of environmental surfaces may play a role in prolonged norovirus outbreaks, the investigation of the environment should be addressed while applying outbreak control interventions [10]. In our case thorough environment investigation of the canteen and the kitchen did not detect any deficiencies. Though, the food was not microbiologically tested, as there were no leftovers and LHA inspectors decided not to take any environmental samples. The noroviruses can also be excreted in the absence of symptoms and incorrect manipulation by an asymptomatic food handler might cause an outbreak [11]. We did not collect any information on which canteen staff prepared the food items that were implicated in the outbreak, or whether they were prepared by the same person. We collected and tested 2 clinical samples of the asymptomatic canteen staff (n = 10), both negative. Still any of the remaining canteen staff might be the source of the outbreak. It is known, that asymptomatic virus shedding may be prolonged in some cases; in immunocompromised patients the virus might be shed even for 20-40 days long [12]. Food may be contaminated by contact with human faecal matter at the source or by unhygienic manipulation by a food handler excreting the virus. This second scenario is probably underestimated because it is difficult to prove [1, 11].

We obtained only 4 stool samples and of these one was positive, therefore one case was classified as confirmed. Some cases were not able to provide the stool samples and for some cases we did not get contact details, although even samples taken later might be valuable as the virus remains in the stool of recovered for 2-3 weeks [7]. Unfortunately, the employees were not easily available for the testing as the outbreak was investigated shortly before the Christmas holiday (Thursday 19th Dec) and at that time many employees were not in the office anymore, as well as during the Christmas time. The detection of viral agents in the clinical and environmental material is usually guite difficult [13, 14]. When it is not possible to get laboratory confirmation of norovirus, health departments can use clinical and epidemiologic criteria to determine that the outbreak was probably caused by norovirus [15]. The original criteria proposed by Kaplan et al [15] are mean illness duration 12 to 60 hrs., mean incubation period 24 to 48 hrs., more than 50% of people vomiting and no bacterial agent found. When all four criteria are present, it is very likely that the outbreak was caused by norovirus. About 30% of norovirus outbreaks do not meet these criteria. If the criteria are not met, it does not mean that the outbreak was not caused by norovirus [15]. Having one confirmed case and considering above mentioned criteria, we concluded the outbreak being of norovirus origin.

Current EU surveillance for foodborne norovirus illness does not capture dispersed outbreaks very efficiently; there is clear evidence of significant underreporting of foodborne norovirus outbreaks [16]. The study of 191 foodborne calicivirus outbreaks in Denmark from 2005 to 2011 [13] demonstrated that in 139 (73%) outbreaks, descriptive epidemiology was the only evidence that food was the vehicle, foodborne transmission was confirmed by the detection of norovirus in food in 20 (11%) outbreaks, samples from the food handler or food handler's family members and from case-patients tested positive for norovirus with identical viral sequences were confirmed in 10 (5%) outbreaks and in 22 (12%) outbreaks, analytical epidemiologic investigations determined a statistical association between illness and suspected food items.

The acute gastroenteritis outbreaks are common worldwide. CZ detects 25–30 food and water-borne outbreaks yearly; in 2017, CZ reported four strong-evidence and 25 weak-evidence food and water-borne outbreaks [3]. Only few of them are published, hardly any internationally, even though some of these were very interesting by findings of by course. In our case, as the population at risk was clearly defined we decided to perform retrospective cohort study, which design is in accordance with other large food-borne norovirus outbreaks in restaurant and similar settings investigations [5, 17, 18]. However, due to laboratory investigation which included only virological testing based on electron microscopy, we might have missed other possible outbreak etiological agents, such as bacteria or toxin. The disease might also not be acquired in the canteen but elsewhere. These hypotheses we are not able to exclude. We would like to emphasize the importance of performing a complete OI, looking at all epidemiological, environmental and microbiological components, when norovirus outbreaks occur [19]. All three are equally important.

Limitations

Limitations of this study are related to the nature of an observational study, recall and selection bias, caused by the data collection through online questionnaire. Questionnaires were sent out quickly, on 19th Dec to NIPH employees and due to technical reasons (time and manner of administration) a day later to SIDC employees. Further, with regard to Christmas holiday, most of the employees were out of the office since 21th Dec at least until 27th Dec. We assume that the response rate would be much higher if we did not experience this delay of sending the link for questionnaire to SIDC employees. Most questionnaires 246 (89.1%) were filled in within 3 days following the outbreak. Therefore, we do not assume significant recall bias that could affect information on the exposure. Selection bias is likely to have influenced our findings, as the questionnaire response rate was low. Due to Christmas holiday we did not send a reminder for online questionnaire. Only 2 out of 10 kitchen staff were tested. There were more food items suspicious and some cases symptoms occurred even before this particular risky lunch. The samples were tested only by electron microscopy.

CONCLUSIONS

Gastroenteritis outbreak among employees of two neighbouring institutions in Prague was early detected and investigated. Epidemic curve demonstrated point source outbreak. Significant association of the food item served on 17th Dec 2019 with occurrence of the gastroenteritis was detected using retrospective analytical cohort study design. This particular vehicle was "Sichuan pork" eaten in combination with "Chinese soup". Only 22 cases were explained by this exposure. Remaining 17 cases could be explained by person to person (airborne) transmission.

We did recommend general control measures including appropriate hygiene and hand-washing routines for food handlers, employees and environmental disinfection in all premises of the canteen, NIPH and SIDC. All employees were informed about the necessity to exclude ill persons from work until they are free from symptoms and to report new gastroenteritis cases to the LHA. The environmental investigation did not detect breaches of food processing of hygiene rules in the canteen. Though this outbreak did not have serious public health consequences, we stress the necessity of complete OI including analytical methods to provide good scientific evidence for further decisions.

The microbiological analysis of one stool sample (NIPH employee) indicated the likely causative pathogen, norovirus. Although there is no direct evidence that the outbreak was caused by norovirus, as we did not have food samples, environmental samples and none positive kitchen staff samples, norovirus still might be the cause of the illness. We recommend in future testing all kitchen staff for common gastrointestinal agents in such an outbreak.

Despite all the limitations, which are common in Ol, and in analytical studies especially, with norovirus which has much cross-contamination, we still found a valid result, so it is a very good example of the technique and the value of these studies. Analytical study is a valid method for providing evidence of association in case there is no microbiological proof of the agent in the vehicle and from the ill cases.

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Conflict of interest

The authors declare that no conflicts of interest exist.

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Ethical approval

Ethical approval was not required as in the Czech Republic public health agencies are able to access and use personal identifiable information for communicable disease outbreak investigations in the public interest. Completion of the questionnaire was considered as implied consent.

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Authors contributions

The authors have all contributed on the outbreak investigation. ML, MS, SB conceived and designed the study, ML and SB performed data analysis and conducted interpretation, ML and MS prepared initial draft of the paper and all authors contributed to the final manuscript preparation. All authors have read and approved the final manuscript.

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