Eurosurveillance
Europe's leading journal on infectious disease epidemiology, prevention and control

Vol. 15 | Weekly issue 28 | 15 July 2010

Rapid communications

Surveillance and outbreak reports

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

Association between 2009 seasonal influenza vaccine and influenza-like illness during the 2009 pandemic: preliminary results of a large household transmission study in Western Australia

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Citation style for this article:
Carcione D, Giele C, Goggin LS, Kwan KS, Smith DW, Dowse GK, Mak DB, Effler P. Association between 2009 seasonal influenza vaccine and influenza-like illness during the 2009 pandemic: preliminary results of a large household transmission study in Western Australia. Euro Surveill. 2010;15(28):pii=19616. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19616

Article published on 15 July 2010

We conducted a prospective household transmission study to examine whether receipt of 2009 trivalent influenza vaccine (TIV) was associated with increased risk of influenza-like illness (ILI) among contacts of confirmed pandemic influenza A(H1N1) 2009 patients. In the week following onset of pandemic illness in a household member, 46 (15%) of 304 TIV-vaccinated contacts, and 174 (15%) of 1,162 unvaccinated contacts developed ILI (p= 0.95). Receipt of 2009 TIV had no effect on one's risk of pandemic illness.

Background

Four recently described observational studies from Canada found that receipt of 2008–09 trivalent influenza vaccine (TIV) was associated with increased risk of pandemic influenza A(H1N1)2009 during the spring and summer of 2009 [1,2]. Determining whether a seasonal influenza vaccine that does not contain pandemic viral antigens can affect one's risk of subsequent infection with the pandemic strain has important implications for public health, as well as our understanding of the immunopathogenesis of influenza infection. Household transmission studies are well-suited to examine this issue because prospective, active follow-up of household contacts can avoid many types of selection biases known to be associated with case-control studies [1].

We conducted a prospective household transmission study during the first ten weeks of the influenza season in Western Australia (29 May–7 August 2009) and examined whether prior vaccination with seasonal 2009 TIV increased the risk of developing influenza-like illness (ILI) among household contacts. Our results should be applicable to the experience in the northern hemisphere as the vaccine recommended by the World Health Organization for use during the 2009 southern hemisphere influenza season was identical to that used in the 2008–09 northern hemisphere influenza season (i.e. A/Brisbane/59/2007 (H1N1)-like virus, A/Brisbane/10/2007 (H3N2)-like virus, and B/Florida/4/2006-like virus) [3,4]

Methods

Index patients were defined as the first symptomatic illness in the household with laboratory-confirmed pandemic influenza A(H1N1) 2009 infection. Interviews with index patients, household contacts, or their carers, established a history of prior vaccination with 2009 TIV. Telephone follow-up with household contacts determined whether they had experienced an ILI in the period beginning at least one day after and within seven days of symptom onset in the index patient. ILI was defined as fever >38°C (or a history of fever when the temperature was not taken) AND cough and/or sore throat. In addition, RT-PCR results on all household contacts with ILI who had a respiratory specimen collected via routine medical follow-up were reviewed. Included in this analysis were 595 households with 1,466 household contacts who had a known 2009 TIV vaccination history (90% of all household contacts). Chi-square tests were used assess statistical differences in proportions; p values <0.05 were considered significant.

Results

Some 304 household contacts reported being vaccinated with 2009 TIV and 1,162 denied vaccination. The proportion of males and females in the vaccinated and unvaccinated cohorts was nearly identical, but the age group distributions differed significantly (Table). Nevertheless, among those with known age, the proportion of vaccinated and unvaccinated household contacts who were 18 years or older was similar (63% and 66%, respectively; p=0.37). Vaccinated household contacts were significantly more likely to report having diabetes or underlying heart, respiratory, or neurological disease (Table). The proportion of vaccinated and unvaccinated household contacts who received antiviral prophylaxis was 13% and 14%, respectively.

A total of 220 (15%) of all household contacts developed ILI within seven days of the onset of illness in the index case; 27 of 29 household contacts with ILI who had a PCR specimen collected within 48 hours of symptom onset were positive for pandemic influenza A(H1N1) 2009 infection. Forty-six (15%) of the 304 TIV-vaccinated contacts and 174 (15%) of the 1,162 unvaccinated contacts developed ILI (p=0.95). When the analysis was restricted to the 941 household contacts aged 18 years or older, the proportion of contacts who developed ILI was 12% in the vaccinated cohort and 13% in the unvaccinated cohort (p=0.86). Prior vaccination with 2009 TIV was not associated with development of ILI among household contacts in logistic regression analyses that simultaneously controlled for age, sex, antiviral prophylaxis, diabetes, heart disease, respiratory disease, and neurological disease (odds ratio:1.0; 95% confidence interval: 0.7 to 1.5; p = 0.95).

Discussion

In Western Australia receipt of 2009 TIV was not associated with increased risk of developing ILI among household contacts of persons with confirmed pandemic influenza A(H1N1) 2009. Conversely, nor was there a protective effect of seasonal 2009 TIV vaccination.

Most investigations in settings outside Canada have found no relationship between 2008–09 TIV and pandemic influenza, but others have reported significant associations, both positive and negative. These studies have employed various methodologies including case-cohort, test-negative case-control, and cross-sectional study designs [5-11]. The discrepant results across these studies most likely reflect differences in the study methods used, but actual variation in the effect of specific vaccines or disparities in the immunological background between populations cannot be discounted [1]. As the largest prospective study to report on this issue to date, our findings make an important contribution to the dialogue regarding the effect of seasonal TIV and the risk of developing pandemic influenza A(H1N1) 2009.

Several limitations of our study should be noted. First, the outcome sought was clinical ILI and not laboratory-proven influenza. Only a small subset of household contacts who developed ILI were tested for influenza; however, a high proportion of the contacts tested by PCR within two days of developing ILI were confirmed as having pandemic influenza A(H1N1) 2009 infection.

Second, vaccination histories were not verified through medical records. Most seasonal 2009 TIV vaccinations would have been administered between the months of March and June 2009, and interviews to determine vaccination status were conducted between late May and early August 2009. Whilst errors in recall may have occurred, it seems reasonable to assume that were this the case such errors would be similar among the cohort who later developed ILI, compared to those who did not.

A strength of our analysis is the ability to simultaneously control for age, antiviral prophylaxis and the presence of underlying medical conditions when examining the association of seasonal TIV and ILI in household contacts.

Table

Demographic characteristics of household contacts of pandemic influenza index cases, by 2009 TIV vaccination status, Western Australia (n=1,466)

	Received 2009 TIV vaccine		
	Yes (n=304) n (%)	No (n=1,162) n (%)	Univariate χ^2
Sex			
Male	150 (49)	597 (51)	0.399[a]
Female	154 (51)	565 (49)	
Age group			
0 to 4 years	44 (14)	105 (9)	<0.001[b]
5 to 17 years	65 (21)	287 (25)	
18 to 50 years	112 (37)	589 (51)	
≥51 years	74 (24)	166 (14)	
Unknown	9 (3)	15 (1)	
Underlying medical conditions			
Diabetes	16 (5)	18 (2)	<0.005[c]
Heart disease	13 (4)	19 (2)	<0.005
Respiratory disease	45 (15)	76 (7)	<0.005
Neurological disease	7 (2)	5 (0)	<0.005

TIV: trivalent influenza vaccine.

[a] Chi-square test for significant difference in sex distribution between vaccinated and unvaccinated household contacts.

[b] Chi-square test for significant difference in age group distribution between vaccinated and unvaccinated household contacts.

[c] Chi-square test for significant difference in specific underlying medical condition between vaccinated and unvaccinated household contacts.

We agree with Janjua and colleagues [12,13] that understanding the potential effect of seasonal TIV on pandemic illness has important ramifications and warrants rigorous investigations that provide sufficient information to assess their validity. The work presented here represents a sub-analysis of data collected as part of a comprehensive assessment of factors that influence the incidence of ILI in household contacts of persons with confirmed pandemic influenza A(H1N1) 2009 infection; the full report containing additional detail on methods and participant characteristics is being prepared. In this interim analysis, we did not find an association, either positive or negative, between 2009 seasonal TIV and subsequent risk of ILI during the 2009 pandemic.

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Impact of emergency oral rabies vaccination of foxes in northeastern Italy, 28 December 2009–20 January 2010: preliminary evaluation

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Citation style for this article:
Capello K, Mulatti P, Comin A, Gagliazzo L, Guberti V, Citterio C, De Benedictis P, Lorenzetto M, Costanzi C, Vio P, Zambotto P, Ferri G, Mutinelli F, Bonfanti L, Marangon S. Impact of emergency oral rabies vaccination of foxes in northeastern Italy, 28 December 2009–20 January 2010: preliminary evaluation. Euro Surveill. 2010;15(28):pii=19617. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19617

Article published on 15 July 2010

Fox rabies re-emerged in northeastern Italy in 2008, in an area bordering Slovenia. In 2009, the infection spread westward to Veneto region and in 2010 to the provinces of Trento and Bolzano. Aerial emergency oral fox vaccination was implemented in the winter 2009-10. Since this vaccination was performed at altitudes below the freezing level, a statistical analysis was conducted to evaluate its impact. Of the foxes sampled following the vaccination campaign, 77% showed a rabies antibody titre of ≥0.5 IU/ml.

Background

In October 2008, rabies infection was diagnosed in a red fox (*Vulpes vulpes*) in the municipality of Resia (Region of Friuli Venezia Giulia, northeastern Italy) [1]. Friuli Venezia Giulia had been affected by rabies in the 1970s and 1980s and, more recently, in the period from 1991 to 1995 [2]. Oral fox vaccination campaigns using SAD B19 vaccine baits [3] were conducted in these areas in 1989 and from 1992 to 2004, and the last documented animal to be infected with rabies was a fox diagnosed in December 1995 in the province of Trieste (Friuli Venezia Giulia), in an area bordering Slovenia. Since then, no other cases had been reported, and since 1997 Italy had been classified as rabies-free.

Following the identification of the infected fox in October 2008, three oral fox vaccination campaigns were conducted in Friuli Venezia Giulia, providing manual distribution of vaccine baits [4]. However, in November 2009, fox rabies spread westward to Veneto region and reached the autonomous provinces of Trento and Bolzano in spring 2010.

Following reports of infection in Veneto region, an emergency vaccination campaign in accordance with the European Union (EU) recommendations [3] was implemented in a large area that included the recently affected regions (Figure 1).

In particular, from 28 December 2009 to 20 January 2010, SAD B19 vaccine baits (Fuchsoral, IDT Biologika) were distributed by helicopter in an area of approximately 9,000 km^2, using a satellite-navigated and computer-supported automatic bait dropping system [5]. An electronic metronome connected to a GPS allowed adjusting the dropping tempo to the speed of the helicopter, permitting an estimated bait coverage of 25-30 baits/km^2. Given that there was no precise information on the size or structure of Italy's fox population, we applied the average bait density recommended for high fox population densities (i.e. 20-30 baits/km^2) [6]. Vaccine baits were distributed only at altitudes below the freezing level (1,000 m above sea level (asl)), taking into account the average winter temperature in the Alps.

Here we provide the results from monitoring the emergency vaccination campaign, in terms of the number of foxes that achieved protective antibody titres [6], and we compare the number of laboratory-confirmed cases of rabies in red foxes in the period before and after the vaccination campaign.

Methodology

The study period ranged from the date that the first case was reported in Veneto region (17 November 2009) to the date of conclusion of the study monitoring the effectiveness of the emergency oral fox vaccination campaign (9 May 2010). The study period was then divided into a pre-vaccination period, taking into account the time needed for the foxes to develop antibodies, and a post-vaccination period. The cut-off date chosen to distinguish these periods was 4 March 2010 (i.e. 30 days after the end of bait distribution). The

tested foxes were those that had been found dead or killed by hunters in the study area (it is mandatory to bring these animals to the National Reference Centre for Rabies at Legnaro Institute).

Case definition

A case of rabies was defined by a positive result in the fluorescent antibody test (FAT) followed by a confirmatory test, i.e. tissue culture isolation (TCIT) or mouse inoculation test (MIT) [6].

The immune response of foxes to oral vaccination was determined by a fluorescent antibody virus neutralisation test (FAVN test) [6]. All analyses were conducted in the laboratory of the National Reference Centre for Rabies at Legnaro Institute.

Results of the winter emergency vaccination campaign

Of 1,917 red foxes tested, 1,324 were collected in the pre-vaccination period and 593 in the post-vaccination period. Rabies was laboratory-confirmed in 170 of the 1,917 foxes (Figure 1). Of these 170, 100 (58.8%) were

Figure 1

Areas with laboratory-confirmed cases of rabies in red fox, northeastern Italy, 17 November 2009–9 May 2010

Source: National Reference Centre for Rabies, Istituto Zooprofilattico Sperimentale delle Venezie – IZSVe, Legnaro (Padova), Italy

Table

Rabies cases by period and altitude at which the fox was found, northeastern Italy, 17 November 2009–9 May 2010 (N=170)

Period	Altitude					
	Below 900 m asl		Above 900 m asl		Total	
	Number	Percentage	Number	Percentage	Number	Percentage
Pre-vaccination	41	41%	59	59%	100	100%
Post-vaccination	11	16%	59	84%	70	100%
Total	52	31%	118	69%	170	100%

asl: above sea level.
Source: National Reference Centre for Rabies, Istituto Zooprofilattico Sperimentale delle Venezie – IZSVe, Legnaro (Padova), Italy.

diagnosed in the pre-vaccination period and 70 in the post-vaccination period. All of the rabid foxes were found dead or shot by hunters in mountainous areas, at altitudes ranging from 398 to 2,224 m asl. The rabid foxes were found at the lower mean altitude (971 m asl) during the pre-vaccination period, compared to the post-vaccination period (1,206 m asl) (one-tailed t-test, p value ‹0.001).

Given that vaccine baits were distributed at altitudes below the freezing level (which corresponds to 1,000 m asl), a conservative cut-off value of 900 m asl was selected to identify the zones covered by vaccination (≤900 m asl) and those not covered (›900 m asl). During the pre-vaccination period, rabies cases were almost equally distributed below and above 900 m asl (41% and 59% of cases, respectively), whereas in the post-vaccination period there was a significantly higher number of cases (84%) found above 900 m asl (chi-square test, p value ‹0.001) (Table). To determine whether this difference was related to vaccination coverage, the homogeneity of the sample in terms of vaccination period and altitude was investigated (the location in which the fox was collected was available for 1,809 of the 1,917 foxes tested). Given that there were no statistically significant differences in the number of foxes when comparing the two periods or the altitudes, the sample was considered to be homogeneous. We can thus hypothesise that the lower number of cases found below 900 m asl during the post-vaccination period was related to vaccination coverage.

With regard to temporal trends, there were no differences in the weekly number of cases by altitude in the pre-vaccination period (Mann-Whitney test, p value=0.078), whereas significant differences were found in the post-vaccination period (p value ‹0.01) (Figure 2).

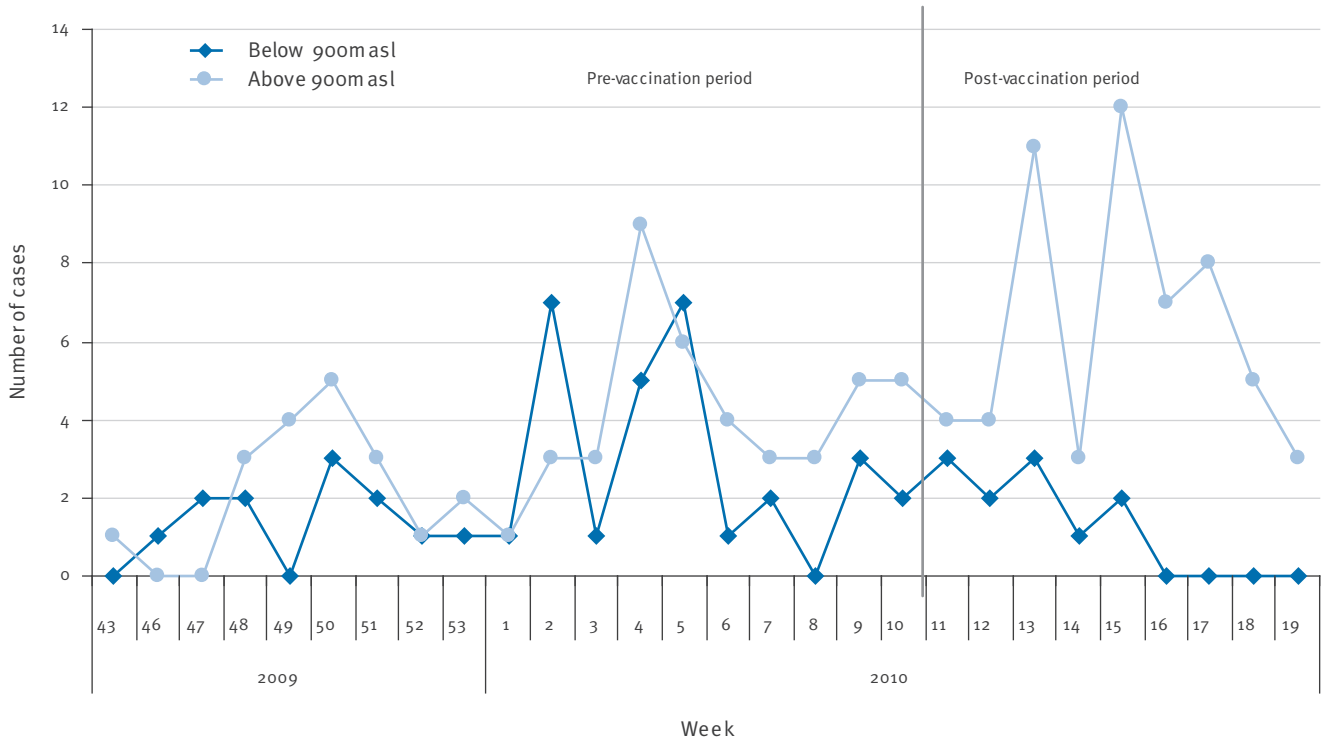
Of the 593 foxes collected in the post-vaccination period, 203 (i.e. those coming from the vaccination area and negative in the FAT assay) were tested by means of FAVN test to investigate the effectiveness of the vaccination campaign. The mean antibody titre was 2.0 IU/ml (min 0.1–max 16.6 IU/ml). Of these 203 foxes, 156 (77%) were considered as immunised, in that their antibody titre exceeded the level considered to be protective by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (≥0.5 IU/ml) [6].

Other rabies control measures

In addition to the emergency vaccination campaign, preventative measures were implemented in the affected areas, including compulsory rabies vaccination of dogs and domestic herbivores at risk of infection (i.e. cows, horses, sheep and goats kept outdoors), movement restrictions of dogs, and enhancement of surveillance in the wild animal population. Furthermore, an information campaign was conducted in order to increase

Figure 2

Weekly trend of laboratory-confirmed cases of rabies by period and altitude at which the fox was found, northeastern Italy, 17 November 2009–9 May 2010 (N=170)



asl: above sea level.

Source: National Reference Centre for Rabies, Istituto Zooprofilattico Sperimentale delle Venezie – IZSVe, Legnaro (Padova), Italy.

risk-awareness of the population and improve preparedness of health services.

Conclusions

The emergency OFV campaign was carried out during the winter, under unfavourable weather conditions. In fact, it is not recommended that vaccination be performed at temperatures below o °C because frozen vaccines do not induce a sufficient immune response and the virus titre may decrease as a result of freezing-thawing cycles [3]. Despite this and the fact that the size and structure of the fox population are unknown, the campaign led to satisfactory immune coverage (77%) and the reduction of rabies incidence below 1,000 m asl. In the spring of 2010, a second aerial OFV campaign was implemented in a larger geographical area (i.e. the entire region of Friuli Venezia Giulia, Trento and Bolzano, and part of the Veneto region). Based on the results of the present monitoring study, this campaign was expanded to cover altitudes up to 2,300 m asl and ended in mid-June.

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Tuberculosis in Greece: bacteriologically confirmed cases and anti-tuberculosis drug resistance, 1995-2009

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Citation style for this article:
Papaventsis D, Nikolaou S, Karabela S, Ioannidis P, Konstantinidou E, Marinou I, Sainti A, Kanavaki S. Tuberculosis in Greece: bacteriologically confirmed cases and anti-tuberculosis drug resistance, 1995-2009. Euro Surveill. 2010;15(28):pii=19614. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19614

Article published on 15 July 2010

The Greek National Reference Laboratory for Mycobacteria is a major source of tuberculosis (TB)-related data for Greece, where the TB burden and epidemiology still need to be better defined. We present data regarding newly diagnosed TB cases and resistance to anti-TB drugs during the last 15 years in Greece. Although the total number of newly detected TB cases has declined, cases among immigrants are increasing. Resistance to first-line anti-TB drugs is widely prevalent, although stable or declining. The implementation of an efficient and effective countrywide TB surveillance system in Greece is urgently needed.

Introduction

Despite remarkable efforts to control tuberculosis (TB), the disease remains prevalent worldwide, and important issues regarding drug resistance have emerged [1,2]. In Greece, according to the recently published report by the European Centre for Disease prevention and Control and the World Health Organization (WHO) Regional Office for Europe, the case notification rate was 6.0 cases per 100,000 population in 2008, while only 37.7% of all reported TB cases were confirmed by bacterial culture [3]. Underreporting is an important problem for TB control in Greece, where various limitations in the national TB monitoring system exist [4]. Reluctance to notify TB cases and failure to collect data at regional and national level makes TB surveillance and trend analysis problematic. Drug-resistant TB is common among repatriated Greeks from the former Soviet Union (FSU; principally Kazakhstan, Russia, Georgia, Uzbekistan, and Kyrgyzstan). Furthermore, migration from regions with high TB incidence (Iraq, Afghanistan, India, Africa etc.) possibly leads to further underestimation of the TB burden and facilitates further spread of the disease.

The Greek National Reference Laboratory for Mycobacteria (NRLM) provides a variety of reference services for both the public and the private healthcare sector in the field of mycobacterial disease. This laboratory constitutes a major source of TB-related data for Greece. Detection, identification and drug susceptibility testing (DST) for first-line anti-TB drugs, are routinely performed for every confirmed case by both conventional and molecular techniques. In addition, DST for second-line drugs is performed for multidrug-resistant (MDR) TB cases.

This report presents the results of the analysis of all culture-confirmed newly diagnosed TB cases referred to the Greek NRLM between 1995 and 2009. A special focus of the report was to document the prevalence of *Mycobacterium tuberculosis* (MTB) resistance against the first-line anti-TB drugs among the native Greek population and immigrants/foreign-born, and especially isoniazid (INH) and rifampicin (RIF), due to their importance for a successful anti-TB treatment.

Methods

We studied 7,042 MTB strains. Only newly diagnosed cases (referred to as 'new cases' in the following) and one sample per patient were included. Patients were categorised as Greeks or immigrants, as declared by the patients themselves on the basis of nationality or, in the case of repatriated Greeks, place of birth. Repatriated Greeks were categorised for the purpose of his study, in the group of immigrants/foreign-born. No ethical approval was required for this study. Ziehl-Neelsen staining, direct microscopy and culture in solid Löwenstein-Jensen medium (LJ) and liquid culture media using initially the Bactec 460 and since 2003 the Bactec MGIT 960 mycobacterial detection systems (Becton Dickinson, Sparks, US) were performed. Bacterial identification was based on conventional phenotypic tests and molecular characterisation using commercially available methods. For first-line drugs, DST was performed in solid LJ (using the proportion method) and MGIT 960 according to the manufacturer instructions. Since 2007, Genotype MTBDRplus (Hain Lifescience, Nehren, Germany) has also been used as a rapid molecular diagnostic tool for MDR-TB detection. For second-line drugs, DST of MDR-TB strains was performed using MGIT 960 during the last five years of the study. Drug concentrations used were according to the WHO guidelines [5].

Table

New tuberculosis cases and resistance rates to anti-tuberculosis drugs during successive five-year time periods, Greece, 1995–2009

Time period	Patients No. (%)			Resistance to anti-TB drugs No. (%)													
				INH (any[a]) No. (%)			RIF (any[a]) No. (%)			MDR No. (%)			XDR No. (%)			XDR:MDR ratio (%)	
	Total	GR	IMM	Total	GR	IMM	Total	GR	IMM	Total	GR	IMM	Total	GR	IMM	Total	GR	IMM
Period A (1995-1999)	**1,955 (28)**	1,613 (82.5)	342 (17.5)	**180 (9)**	112 (7)	68 (20)	**79 (4)**	47 (3)	32 (9)	**71 (4)**	39 (2.5)	32 (9)	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
Period B (2000-2004)	**2,798 (40)**	2,057 (73.5)	741 (26.5)	**245 (9)**	168 (8)	77 (10)	**110 (4)**	75 (4)	35 (5)	**88 (3)**	61 (3)	27 (4)	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
Period C (2005-2009)	**2,289 (32)**	1,339 (58.5)	950 (41.5)	**154 (7)**	73 (5.5)	81 (8.5)	**90 (4)**	36 (3)	54 (6)	**69 (3)**	26 (2)	43 (4.5)	**13 (0.6)**	9 (0.7)	4 (0.4)	**19%**	35%	9%
Total (1995-2009)	**7,042**	**5,009 (71)**	**2,033 (29)**	**579 (8)**	**353 (7)**	**226 (11)**	**279 (4)**	**158 (3)**	**121 (6)**	**228 (3)**	**126 (2.5)**	**102 (5)**	**13 (0.6)**	**9 (0.7)**	**4 (0.4)**	**19%**	**35%**	**9%**

GR: Greeks; IMM: immigrants; INH: isoniazid; MDR: multidrug-resistant; n.d.: not determined; RIF: rifampicin; TB: tuberculosis; XDR: extensively drug-resistant.

[a] Any phenotypes that include this resistance, not exclusively monoresistance.

[b] Definition not applicable before 2006, when the latest WHO definition of XDR-TB used here was agreed.

Differences in resistance rates were compared using the chi-square test. Statistical significance was set at 95%. Odds ratios and coefficients of determination (R^2= the square of the sample coefficient) were also calculated. Statistical analyses were performed using GraphPad Prism software v.5 (GraphPad, San Diego, US).

Results

Among 7,042 new TB cases, 5,009 (71%) were Greeks and 2,033 (29%) were immigrants/foreign born (Table). The total number of new cases per year increased from 320 in 1995 to a peak of 650 in 2000, and has since then steadily declined to 441 cases in 2009 (Figure 1). New cases among Greeks followed a similar trend. During the same period, the total number of new cases in immigrants increased almost sixfold from 40 cases (1995) to 240 (2009). In fact, 2009 was the first year when the absolute number of new cases among immigrants/foreign born was higher than among Greeks (240 versus 201 cases, respectively). New cases among immigrants accounted for 54.4% of total TB cases in 2009 (compared with 12.5% in 1995).

In total, 6,130 of the 7,042 TB isolates (87%) were found sensitive to all anti-TB drugs; 912 isolates (13%) were resistant to at least one drug (Figure 2). Drug resistance data for INH, RIF, MDR-TB and extensively drug-resistant (XDR)-TB during three successive time periods of five years (A: 1995-1999; B: 2000-2004; C: 2005-2009) are also presented in the Table. Resistance to INH decreased among Greeks (8% in period B to 5.5% in period C), a statistically significant difference (p=0.0026). The same applies to the immigrants group, with a decrease from 20% in period A to 8.5% in period C (p<0.0001). Resistance to RIF remained constant among Greeks during the whole time period (p=0.7124), while it dropped from 9% (period A) to 6% (period C) among immigrants (p=0.0195). Resistance to INH or RIF was more likely to be found in the immigrants group during the whole time period of the report. The MDR-TB rate (2.5-3%) remained constant among Greeks (R^2=0.0037; p=0.3802), while it decreased from 9% (period A) to 4.5% (period C) among immigrants (R^2=0.1484; p=0.0011). MDR-TB was 2.04 more common in immigrants compared to Greeks (OR: 2.05; 95% CI: 1.57–2.67) (Figure 3).

Mono-resistance to streptomycin (SM) was detected in 245 isolates (26% of resistance phenotypes; 3.5% of cases), and this was the most frequent resistance phenotype followed by mono-resistance to INH (148 isolates; 16% of phenotypes; 2% of cases), combined resistance to SM+INH (136 isolates; 15% of phenotypes; 1.9% of cases) and resistance phenotype SM+INH+RIF+Ethambutol (EMB) (84 isolates; 9% of phenotypes; 1.2% of cases). Combined resistance to all first-line anti-TB drugs was found in 26 isolates (3% of phenotypes; 0.36% of all cases). Mono-resistance to RIF was rather uncommon (n=33; 4% of phenotypes; 0.46% of new cases), confirming a RIF mono-resistance

rate of 0.38% previously reported [6]. Finally, since the year 2006, 13 XDR-TB cases (0.6%) have been recorded in the Greek NRLM, nine (0.7%) cases among Greeks and four (0.4%) cases among immigrants (p=0.8798).

Discussion

In the period under investigation, and according to the 2001 census, the population of Greece increased from 10,259,900 in 1991 to 10,964,020 in 2001 [6]. It is estimated that this increase is attributable almost entirely to immigration, with 762,191 'foreigners' living

Figure 1

Bacteriologically confirmed new tuberculosis cases per year, Greek National Reference Laboratory for Mycobacteria, 1995–2009

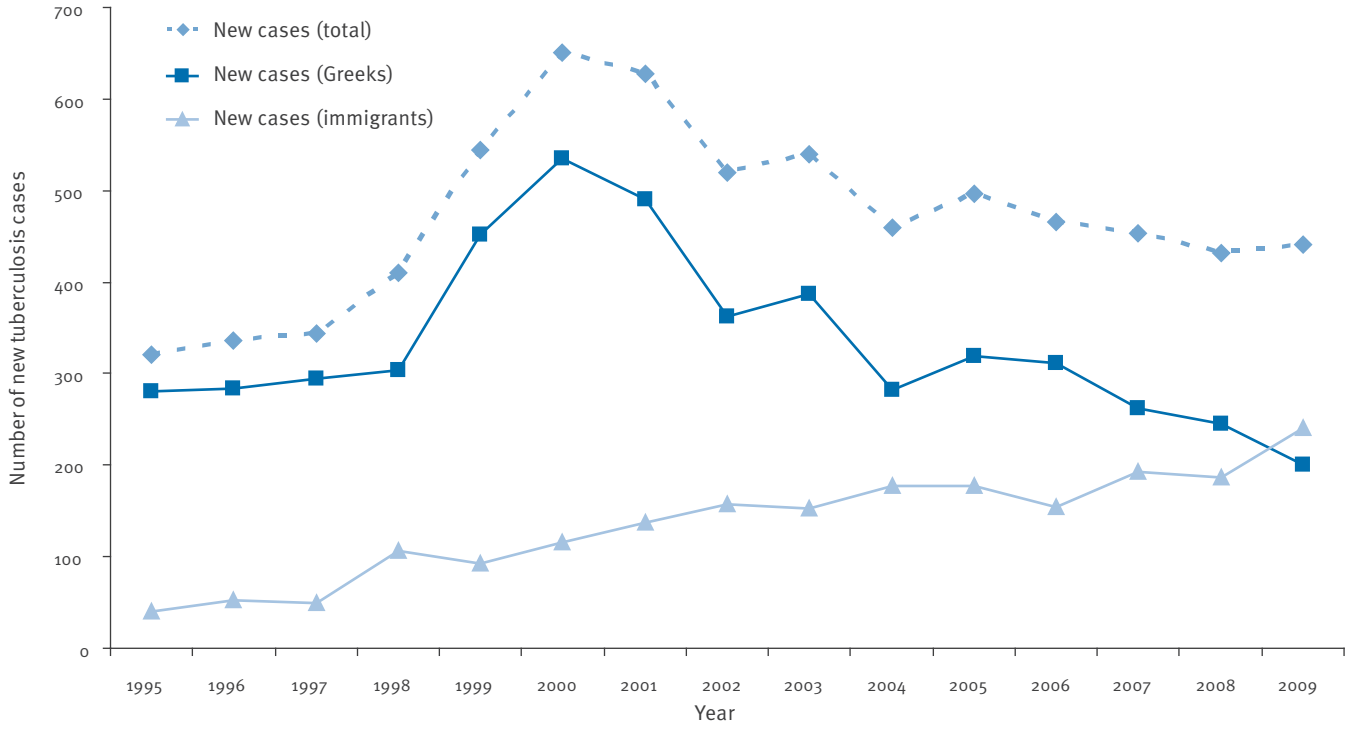
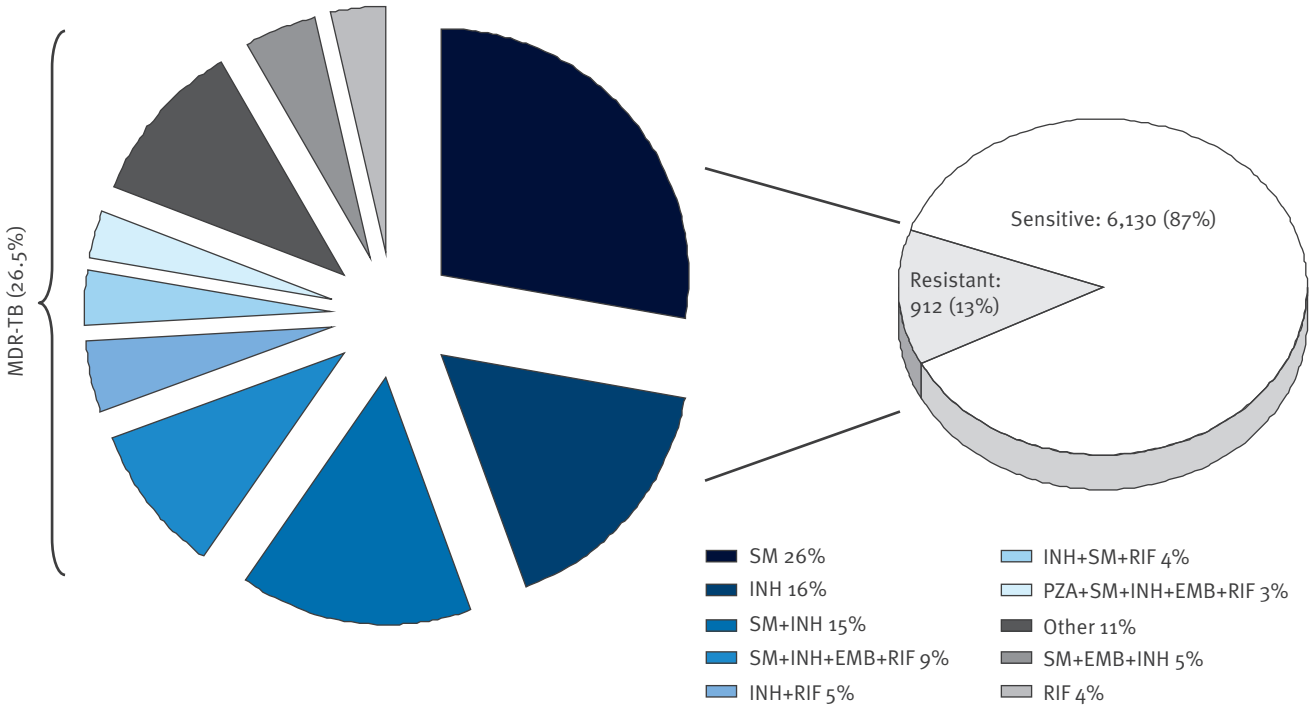


Figure 2

Drug susceptibility testing data and tuberculosis resistance phenotypes rates, Greece, 1995-2009



EMB: ethambutol; INH: isoniazid; MDR: multidrug-resistant; PZA: pyrazinamide; RIF: rifampicin; SM: streptomycin; TB: tuberculosis.

in Greece in 2001, approximately 7% of total population [6]. Greece has received several hundred thousands of immigrants from countries with a higher TB prevalence. In 1998, the implementation of country-wide immigrants' health inspection programmes contributed to the better TB management. After a sharp increase in TB incidence in 2000, possibly connected to a peak of immigration from the FSU (including repatriated Greeks) and to better case reporting, new TB cases per year in total and among Greeks decreased. As documented by this report and also by Kanavaki *et al*. [7], the number of new TB cases recorded among immigrants has increased sixfold during the past 15 years. A similar trend has previously been reported for pooled data from other European countries [8]. In line with this trend, the present study found that in 2009, the absolute number of new cases among immigrants/foreign born was for the first time higher than among Greeks. This trend could be attributed to several factors, including improved accessibility to healthcare services for immigrants. However, the degree of undernotification of TB cases remains high, leading to substantial underestimation of the disease burden in Greece [4].

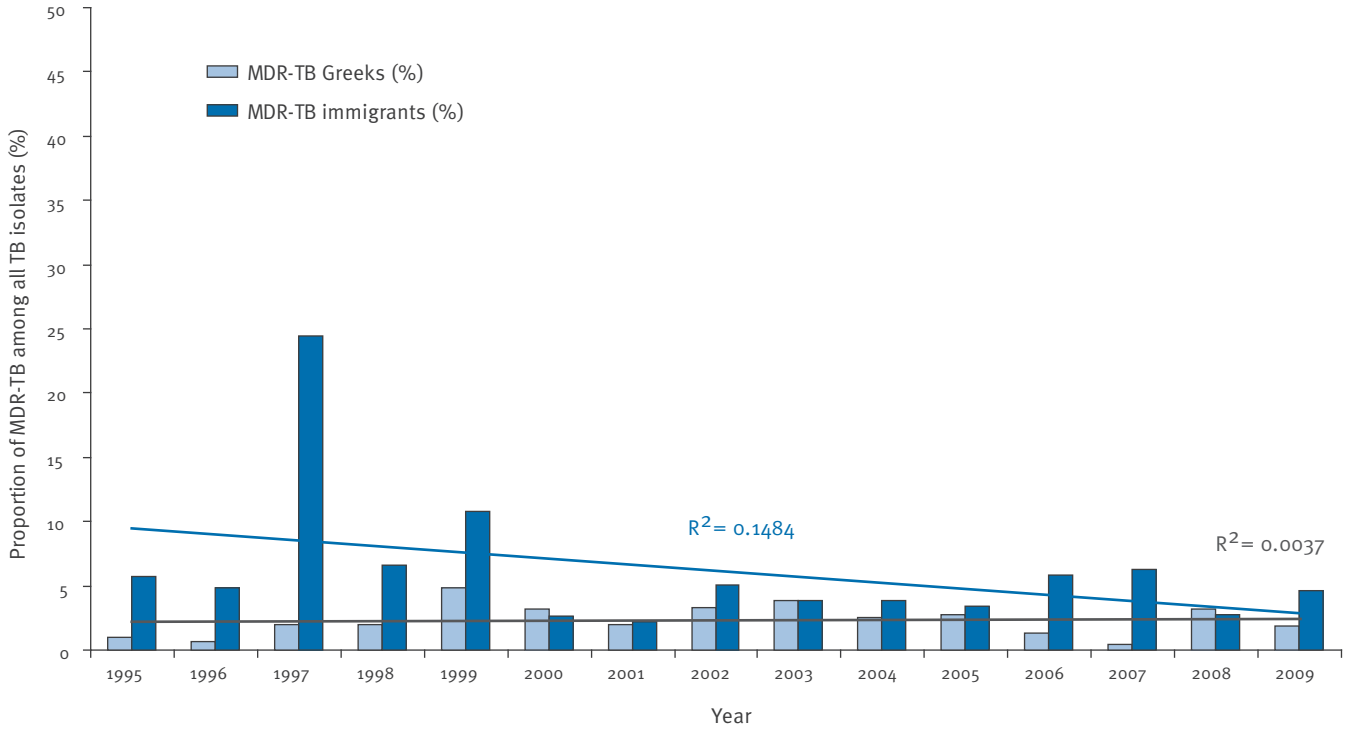
Another important finding of the study was the decreasing prevalence of MTB resistant to INH in both study groups. From period A to period C, the prevalence of RIF-resistant and MDR-TB strains decreased by almost half among immigrants, which could be attributed to the fact that immigration to Greece from the FSU states, where RIF resistance and MDR-TB was highly prevalent, culminated in the early 1990s [6]. However, previous [7] and current data confirm that the absolute number of resistant cases in immigrants still appears to be increasing.

Resistance to first-line anti-TB drugs remains higher in Greece than in most other countries in western Europe [9,10]: Resistance to INH was slightly higher in Norway (10.4%), Sweden (9.9%), Austria (9.5%) and Luxemburg (8.3%). Much higher INH resistance rates were observed in FSU countries, for example: Kazakhstan (42.6%), Latvia (30.9%), Russia (26%), Georgia (23.4%), Estonia (20.6%), and Lithuania (20.3%). RIF resistance was also higher in Greece (4%) compared with other western European countries. MDR-TB was much higher in Kazakhstan (14.2%), Estonia (13.3%), Russia (12.5%), Latvia (10.8%), Lithuania (9.8%) and Georgia (7.6%) than in Greece (4%) and the rest of Europe (median European MDR rate 1.0%) [10]. Knowledge of the epidemiology of resistance patterns in a country is critical for the introduction of national guidelines for the management of tuberculosis. Streptomycin mono-resistance appears to be very common in Greece. Data regarding second-line drugs are limited and should be interpreted with caution.

The current report has several limitations. Firstly, the representativeness of the sample is unknown. Sampling bias, especially among immigrants/foreign born, could not be excluded. Although access of the immigrant population to the national healthcare system has improved over the years, many patients among

Figure 3

Percentage of multidrug resistance, Greeks versus immigrants/foreign-born, Greece, 1995-2009



MDR: multidrug-resistant; TB: tuberculosis.

foreigners living in Greece do not have full access to hospital services. In addition, repatriated Greeks from the FSU could not always be clearly identified within the native Greek population. Secondly, incomplete patient records, lacking data on epidemiological risk factors (such as belonging to a vulnerable group, previous treatment status, sex and age), and the lack of genotyping data for MTB isolates, limited the estimation of resistance transmission among the study population. The implementation of a nationwide TB Genotyping and Surveillance Network will be essential for tuberculosis control in Greece.

Acknowledgements

The authors would like to thank technicians Spyridoula Anagnostou, Paraskevi Karfi, Maria Kolettou, Kyriaki Toumbaniari, Ekaterini Raftopoulou, Marina Panagi, and Anastasios Skouroglou for their technical assistance and also all colleagues who worked in the lab during the last 15 years.
Updated data regarding new TB cases and anti-TB drugs resistance in the NRLM can be accessed via the Greek System for the Surveillance of Antimicrobial Resistance (WHONET GREECE) webpage at http://www.mednet.gr/whonet/. We would like to thank Professor Alkiviadis Vatopoulos and Mr Michael Polemis for their support.
This report was funded internally by the Sotiria Chest Diseases Hospital, Athens, Greece.

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Outbreak investigation in two groups of coach passengers with gastroenteritis returning from Germany to the Netherlands in February 2009

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Citation style for this article:
Visser H, Verhoef L, Schop W, Götz HM. Outbreak investigation in two groups of coach passengers with gastroenteritis returning from Germany to the Netherlands in February 2009. Euro Surveill. 2010;15(28):pii=19615. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19615

Article published on 15 July 2010

In February 2009, an outbreak of 38 cases of gastroenteritis occurred among the participants of two Dutch coach trips (A and B) who visited the same hotel in Germany. We initiated an outbreak investigation to determine possible risk of food-borne infection. A retrospective cohort study was performed among 87 passengers using a self-administered questionnaire. The response rate was 75 of 87 (86%). Mean age was 65 years. Cases were defined as participants of the two coach trips who had diarrhoea and/or vomiting at least once within 24 hours in the period between 7 and 14 February 2009. We distinguished early and late cases, with symptoms starting within or after 72 hours of arrival in the hotel. Overall attack-rate was 38 of 75 (51%). Microbiological investigation was performed on stool samples of two passengers from Coach A and two passengers from Coach B. Identical norovirus genotype II.4 sequences were detected in all four samples. Univariate analysis revealed a potential risk for early cases from juice consumption , which was most clearly seen for Coach B on day of arrival (juice at lunch: relative risk (RR): 3.9, 95% confidence interval (CI): 1.3–11.7; juice at dinner: RR: 5.5, 95% CI: 1.6–18.1). A dose-response relationship was found. This outbreak was probably caused by using the taps of juice served in large containers with a tap for self-service, due to environmental contamination through person-to-person transmission. Still the role of either contaminated juice or contact with contaminated juice cannot be ruled out.

Introduction

Noroviruses are a common cause of gastroenteritis outbreaks with an incubation period of 12 to 72 hours. The characteristic symptoms of vomiting and diarrhoea are short-lived, lasting two to three days, but can last longer in older and vulnerable individuals (such as hospitalised persons or residents of nursing homes) [1]. Symptomatic humans can shed the virus as early as several hours before onset of symptoms until three weeks after recovery. Asymptomatic shedding has been reported [2,3]. Transmission of the virus can occur through contact with infected persons, contaminated environment or contaminated aerosols, as well as through consumption of contaminated food or water. Given the low dose needed for infection [4] and the fact that person-to-person transmission quickly takes over with high attack rates (30-60%), identification of a point source of infection during an outbreak is complicated. Moreover, food or water contaminated by noroviruses usually appears to be in good condition and detection of norovirus in food or water is complicated because viruses do not replicate outside their host and are therefore present only in small numbers in the food. Contamination of food can occur at any point during production, preparation and handling of food, including the preparation of individual servings by infected food handlers [5].

On 9 February 2009, the Municipal Public Health Service (MPHS) Rotterdam-Rijnmond was notified of several passengers who had developed symptoms of diarrhoea and/or vomiting within a short time period during a two-day coach trip to Germany. On the evening of 11 February 2009, an outbreak in a second coach was notified, triggering an outbreak investigation. At that time, six passengers were admitted to a hospital in the Netherlands. Both coaches had followed the same 3,5-hour travel itinerary, and the two groups of passengers had stayed in the same hotel in Germany for consecutive periods of two days, indicating a potential common source of infection. We investigated the outbreak to determine its size and to identify a potential common food-borne source in order to implement measures limiting further spread to other groups of visitors.

Materials and methods

Laboratory analysis

We obtained stool samples from four patients who were admitted to the emergency ward, two from each coach, and they were tested by norovirus ELISA (RIDASCREEN, R-Biopharm AG) in a regional diagnostic laboratory. We used the diagnostic algorithm for outbreaks of gastroenteritis used in the Netherlands [6]. The four

positive samples were sent to the National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) for further confirmation of the causative agent using RT-PCR and subsequent sequencing of the capsid gene (VP1) for genotyping and comparison of strains [7,8].

Epidemiological investigation

Outbreak description

Coach A left the Netherlands on 7 February 2009 and returned in the evening of 9 February. Coach B left on 9 February and returned in the evening of 11 February. Itinerary, accommodation and meals served in the hotel were identical for both coach trips. Passengers from Coach A and Coach B were not in contact with each other. The first notification was made to the MPHS Rotterdam-Rijnmond on 9 February 2009.

Data collection

The MPHS Rotterdam-Rijnmond contacted the organising travel agency, and subsequently the local German public health office (Gesundheitsamt), to obtain the passenger list and information on the meals served in the hotel during the trips. As the menu appeared to be unavailable, we performed hypothesis-generating interviews with six randomly chosen listed passengers and the four passengers whose stool had been investigated, in order to assess the food items served during their stay in the hotel. In addition, the 10 passengers were interviewed using a trawling questionnaire for other details of the journey. Because some of the passengers were resident outside of the Rotterdam-Rijnmond region, an outbreak alert was sent to all MPHSs in the Netherlands. The MPHSs were asked to report any cases linked to the outbreak. We performed a retrospective cohort study among the 87 passengers of both coaches. Demographic data, history of gastroenteritis, consumed food items and possible exposures such as contact with ill persons and which toilets were used during the journey (onboard toilet and toilet use during stops) and during the stay in the hotel (toilet use in the hotel and during excursions) were assessed using self-administered questionnaires.

Data analysis and case definition

The cases were defined as travellers on Coach A or B who had diarrhoea and/or vomiting at least once within 24 hours in the period between 7 and 14 February 2009. To be able to identify a potential common food-borne source of infection, we analysed early and late cases separately in order to distinguish a potential initial source of infection from later person-to-person transmission. Early cases were defined as those with symptom onset within 72 hours after arrival in the hotel, late cases as those with symptom onset later than 72 hours after arrival in the hotel. In a sub-analysis for significant risk factors, we considered a case definition including biological plausibility for a suspected food item or other risk factor to be the source of infection: a biologically plausible case was defined

Figure 1

Steps in the outbreak investigation, February 2009

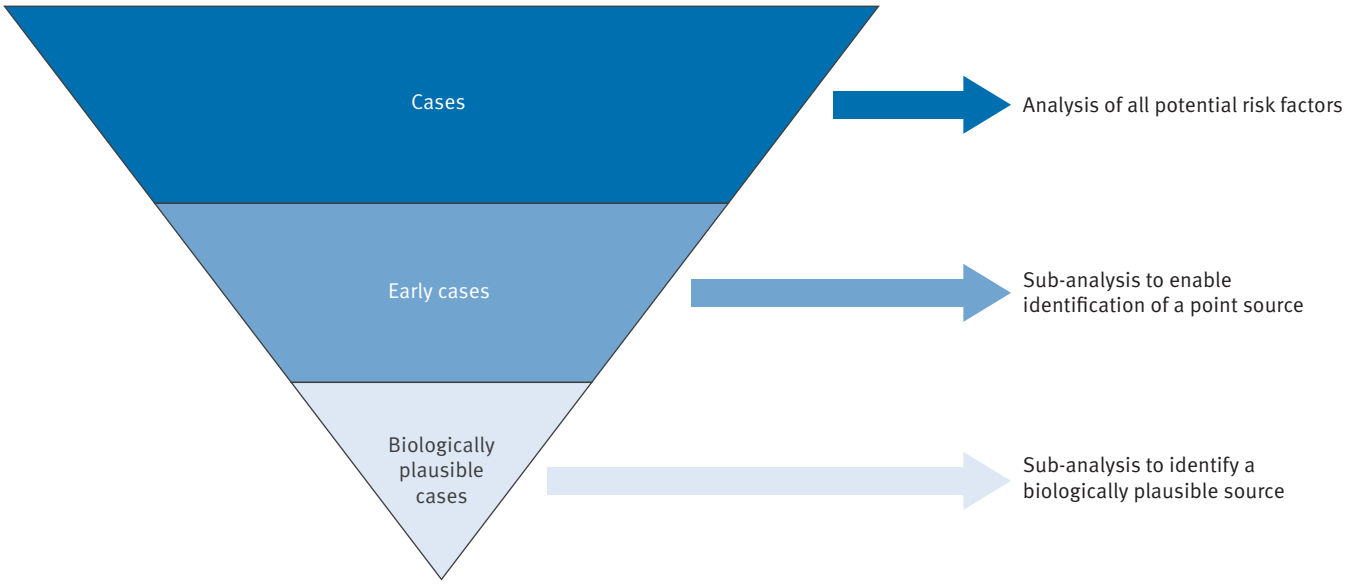


Table 1

Demographics of passengers of Coaches A and B, norovirus outbreak, February 2009 (N=75)

	Male			Female			Total		
	Number	%	Mean age (years)	Number	%	Mean age (years)	Number	%	Mean age (years)
Coach A	15	44	67	19	56	62	34	100	64
Coach B	12	29	63	29	71	66	41	100	65

as a case showing symptoms within 72 hours after consumption of that specific food item or exposure to that specific risk factor (Figure 1). We analysed the data for Coach A and Coach B independently as the two passenger groups had not been in contact with each other. Although the travellers were served identical meals, the food items were not exactly the same for both trips. Therefore we considered it inadequate to analyse the data for the whole group together.

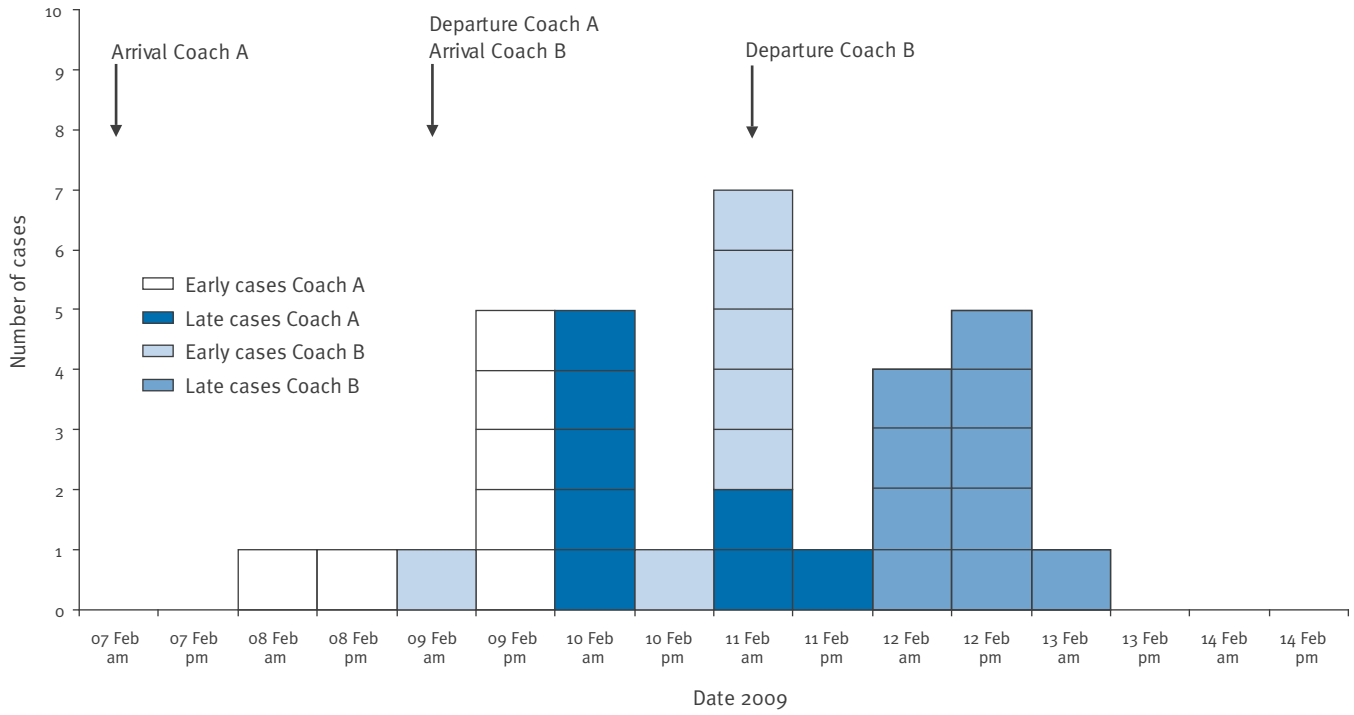
Univariate analysis was performed, calculating relative risks (RR) and 95% confidence intervals (CI) for dichotomous individual exposures for becoming either a case or an early case in separate analysis. In addition, logistic univariate analysis was used to calculate point estimates including their 95% CI for discrete variables of consumption frequencies. Multivariate analysis was performed, including the variables that were found to be significant during univariate analysis, with variables, such as age, treated as continuous where possible. The variables remained in the model if p values were <0.10, while the backward selection procedure was used. All analyses were stratified for Coach A and B. Statistical analyses were performed using Microsoft Excel, SPSS version 15.0 and SAS version 9.1 for Windows.

Environmental investigation and prevention measures

On 12 February, we informed the local German public health office where the hotel was situated, and requested an environmental investigation in the hotel. The travel agency was provided with Dutch guidelines for hygiene measures in settings with successive passenger groups [9]. The passengers were provided with information, attached to the questionnaire, about norovirus infections and how to prevent further transmission.

Figure 2

Early and late cases by onset of symptoms, norovirus outbreak, February 2009 (N=32)



Date and hour of disease onset was available for 32 of 38 persons.

Table 2

Hotel menus with description of food items, norovirus outbreak, February 2009

Day 1							
Lunch	Soup	Bread			Juice	Coffee	Tea
Dinner	Macaroni	Chicken/Turkey	Cabbage		Juice	Coffee	Tea
Days 2 and 3							
Breakfast	Bread	Cheese	Egg	Jam	Juice	Coffee	Tea
Day 2							
Lunch	Chicken	Rice	Carrots		Juice	Coffee	Tea
Dinner	Potatoes with bacon	Pork chop	Carrots in sauce		Juice	Coffee	Tea

Results

Laboratory Analysis

With four of four patient samples testing positive for norovirus in both ELISA and RT-PCR, this outbreak could be attributed to norovirus as the causative agent [10]. Sequencing of the RT-PCR fragments obtained from the four samples resulted in a full capsid sequence of ca. 1,600 nt, two partial capsid sequences of ca. 600 nt and one partial capsid sequence of ca. 300 nt. All four sequences belonged to the norovirus genogroup II.4 variant 2006b, and had 100% identity.

Epidemiological investigation

Descriptive epidemiology

Of 87 listed passengers, 75 returned a filled questionnaire (response rate 86%). Of these 75 passengers, 48 (64%) were female and 27 (36%) were male, with a mean age of 65 years for both sexes.

A total of 39 people met our case definition. One of these reported to have chronic diarrhoea and was therefore not considered to be a case, leaving 38 cases for analysis. The overall attack rate was 38 of 75 (51%), with attack rates of 18 of 34 (53%) and 20 of 41 (49%) for Coach A and B, respectively. The attack rate for men and women in Coach A was 53% and 53%, respectively (RR: 1.0, 95% CI: 0.5–1.9), and for Coach B 48% and 50%, respectively (RR: 1.0, 95% CI: 0.5-1.9). As the attack rate for men and women was not different, we did not include sex as confounder in the data analysis.

Among the 38 cases, 28 persons had at least two episodes of diarrhoea and/or vomiting within 24 hours, and 10 persons reported one such episode, combined with nausea and watery diarrhoea, fever higher than 38 °C, bowel cramps, headache and loss of appetite. The combinations of complaints were strongly indicative of a norovirus infection and therefore these people were included as cases. Nine of the 38 cases (24%) reported slimy diarrhoea, 27 (71%) reported watery diarrhoea, 13 (34%) fever, 17 (45%) stomach cramps, 29 (76%) nausea and 32 (84%) loss of appetite. Six passengers

Table 3

Analysis of food intake for all cases (A) and for early cases (B) in Coach A (N=34[a])

A

	Exposed			Not exposed					
Coach A (early and late cases)	Ill	Not ill	AR exposed	Ill	Not ill	AR unexposed	RR	95% CI	% cases exposed
Day 1 lunch	18	14	56%	0	2	0%	NA	NA	100%
Day 1 juice at lunch	5	3	63%	13	12	52%	1.20	0.62-2.32	28%
Day 1 dinner	17	15	53%	1	1	50%	1.06	0.26-4.41	94%
Day 1 juice at dinner	3	4	43%	14	11	56%	0.77	0.30-1.93	18%
Day 2 breakfast	17	13	57%	1	3	25%	2.27	0.40-12.73	94%
Day 2 juice at breakfast	11	7	61%	7	8	47%	1.31	0.68-2.52	61%
Day 2 lunch	17	13	57%	1	3	25%	2.27	0.40-12.73	94%
Day 2 juice at lunch	1	3	25%	17	11	61%	0.41	0.07-2.31	6%
Day 2 dinner	15	11	58%	3	5	38%	1.54	0.59-3.99	83%
Day 2 juice at dinner	5	3	63%	13	12	52%	1.20	0.62-2.32	28%
Day 3 breakfast	16	15	52%	2	1	67%	0.77	0.32-1.85	89%
Day 3 juice at breakfast	7	6	54%	11	9	55%	0.98	0.52-1.86	39%

B

	Exposed			Not exposed					
Coach A (early cases)	Ill	Not ill	AR exposed	Ill	Not ill	AR unexposed	RR	95% CI	% cases exposed
Day 1 juice at lunch	4	4	50%	6	17	26%	1.92	0.72-5.09	40%
Day 1 dinner	10	20	33%	0	2	0%	NA	NA	100%
Day 1 juice at dinner	1	4	20%	9	16	36%	0.56	0.09-3.46	10%
Day 2 breakfast	10	18	36%	0	4	0%	NA	NA	100%
Day 2 juice at breakfast	7	10	41%	3	11	21%	1.92	0.61-6.09	70%
Day 2 juice at lunch	1	3	25%	9	17	35%	0.72	0.12-4.27	10%
Day 2 dinner	7	17	29%	3	5	38%	0.78	0.26-2.31	100%
Day 2 juice at dinner	3	4	43%	7	17	29%	1.47	0.51-4.23	30%
Day 3 breakfast	9	20	31%	1	2	33%	0.93	0.17-5.04	90%
Day 3 juice at breakfast	5	7	42%	5	14	26%	1.58	0.58-4.33	50%

AR: attack rate; CI: confidence interval; NA: not applicable; RR: relative risk.

[a] Per food-item, the denominator varies. Answers were not given for all items on the questionnaires.

(16%) were admitted to hospital in the Netherlands. All recovered without sequelae.

The epidemic curve (Figure 2) shows the cases by date of onset of symptoms with a 12 hour interval, with distinction between the early and late cases for each of the two coaches. In both Coach A and Coach B, the initial cases occurred 24 hours after arrival in the hotel. The majority of the people in Coach B developed symptoms after departure from the hotel. A rapid drop in the incidence of new cases within the group of coach travellers is visible after arrival in the Netherlands when the group split up.

Data analysis

The menu served on day 1, day 2 and day 3 are listed in Table 2 and were identical on both trips. We investigated the food items grouped together in meals as well as individual components of the meals for each coach trip separately, to see if there was an association between consumption and being a case in general or an early case.

Univariate analysis of the passengers from Coach A did not indicate a significant food-borne source for either cases in general (Table 3A) or early cases (Table 3B). However, illness of the early cases in Coach A was suggestive of being associated, albeit not significantly, with drinking juice on day 2 at breakfast (RR: 1.92; 95% CI: 0.6–6.1), day 2 at dinner (RR: 1.47; 95% CI: 0.5–4.2) and day 3 at breakfast (RR: 1.58; 95% CI: 0.6–4.3).

As shown in Table 4, univariate analysis in the passengers from Coach B showed a significant association only with drinking juice. This is most obvious among the early cases (Table 4B), who had a strong association with drinking juice on day 1 at lunch (RR: 3.88; 95% CI: 1.3–11.7), day 1 at dinner (RR: 5.45; 95% CI: 1.6–18.1) and a less strong association for day 2 at dinner (RR: 3.02; 95% CI: 1.0–9.4).

Table 4

Analysis of food intake for all cases (A) and for early cases (B) in Coach B (N=41[a])

A

	Exposed			Not exposed					
Coach B (early and late cases)	Ill	Not ill	AR exposed	Ill	Not ill	AR unexposed	RR	95% CI	% cases exposed
Day 1 lunch	19	21	48%	1	0	100%	0.48	0.34-0.66	95%
Day 1 juice at lunch	6	4	60%	14	17	45%	1.33	0.70-2.51	30%
Day 1 dinner	10	1	91%	10	20	33%	2.73	1.59-4.68	95%
Day 1 juice at dinner	18	18	50%	2	3	40%	1.25	0.41-3.84	50%
Day 2 breakfast	14	11	56%	6	10	38%	1.49	0.73-3.07	90%
Day 2 juice at breakfast	18	18	50%	2	3	40%	1.25	0.41-3.84	70%
Day 2 lunch	6	3	67%	14	18	44%	1.52	0.83-2.79	90%
Day 2 juice at lunch	9	3	75%	11	18	38%	1.98	1.12-3.49	30%
Day 2 dinner	20	20	50%	0	1	0%	NA	NA	95%
Day 2 juice at dinner	9	8	53%	11	13	46%	1.16	0.62-2.16	45%
Day 3 breakfast	19	20	49%	1	1	50%	0.97	0.23-4.04	100%
Day 3 juice at breakfast	19	20	49%	1	1	50%	0.97	0.23-4.04	45%

B

	Exposed			Not exposed					
Coach B (early cases)	Ill	Not ill	AR exposed	Ill	Not ill	AR unexposed	RR	95% CI	% cases exposed
Day 1 juice at lunch	5	5	50%	4	27	13%	3.88	1.28-11.70	56%
Day 1 dinner	8	31	21%	1	1	50%	0.41	0.09-1.87	89%
Day 1 juice at dinner	6	5	55%	3	27	10%	5.45	1.64-18.14	67%
Day 2 breakfast	9	27	25%	0	5	0%	NA	NA	100%
Day 2 juice at breakfast	8	17	32%	1	15	6%	5.12	0.71-37.15	89%
Day 2 juice at lunch	3	6	33%	6	26	19%	1.78	0.55-5.74	33%
Day 2 dinner	9	30	23%	0	2	0%	NA	NA	100%
Day 2 juice at dinner	5	7	42%	4	25	14%	3.02	0.98-9.35	56%
Day 3 breakfast	9	31	23%	0	1	0%	NA	NA	100%
Day 3 juice at breakfast	5	12	29%	4	20	17%	1.76	0.55-5.62	56%

AR: attack rate; CI: confidence interval; NA: not applicable; RR: relative risk.

[a] Per food-item, the denominator varies. Answers were not given for all items on the questionnaires.

When considering biological plausibility and dose of juice consumption associated with cases or early cases, we considered the number of meals at which juice was consumed each day, with a maximum of two for day 1, three for day 2, and one for day 3. As the exact time of onset of symptoms was only known for 32 of 38 cases, we chose to use an onset of symptoms within four days after consumption to make sure the maximum incubation period of 72 hours was included. In Coach A, the risk from juice consumption increased per day, although non-significantly, with 1.4 (95% CI: 0.4–4.8) for day 1, 1.6 (95% CI: 0.6–3.7) for day 2, and 2.0 (95% CI: 0.4-10.5) for day 3. In contrast, a decreasing and significant association was seen per day for coach B, with 5.0 (95% CI: 1.6–15.0) for day 1, 2.4 (95% CI: 1.0–5.6) for day 2 and 1.3 (95% CI: 0.2–6.6) for day 3. Both groups experienced highest risk from consumption of juice served in a container on 9 February.

To assess whether visiting the toilet on board the coach during the return trip was a risk for becoming a case, biological plausibility was again considered, now by excluding the cases who had become ill before departure from the hotel. There appeared to be no significant risk of illness after using the onboard toilet (data not shown).

Despite low numbers, stratified multivariate logistic regression was performed to determine whether the univariate model could be improved. The backward selection model included the number of times juice was consumed on days 1, 2 and 3, lunches and dinners on days 1 and 2, breakfast on days 2 and 3, use of toilets in the hotel restaurant, coach and elsewhere during stops, and age in years. The analysis resulted in an invalid model which did not improve the univariate model. However, the results were consistent with univariate analysis, indicating a risk from juice consumption on 9 February in both groups.

Prevention measures

The German public health office performed an environmental health investigation in the hotel. Personal communication between the German and Dutch authorities revealed several critical points in the hygiene procedures. Both the MPHS Rotterdam-Rijnmond and the RIVM tried to obtain the results of the environmental investigation from the German public health office, but were informed that results of environmental investigations are not available to the human infectious disease unit due to regulations. Both coaches were thoroughly cleaned according to Dutch guidelines, and not directly used for successive passenger groups. No subsequent outbreaks or cases were notified from the travel agency. In addition, no additional secondary cases were reported after the groups split up in the Netherlands, breaking the transmission chain.

Discussion and conclusion

In this paper we show that consumption of juice may have contributed to an outbreak of norovirus infections among two cohorts of travellers following the same itinerary within a short period of time. We decided not to consider the two groups as one. Sub-analysis showed that the results were less conclusive, indicating that the two groups were different. The two groups of travellers had not been in contact with each other, did not share the same coach, and none of the cases had been in contact with persons who showed signs of gastroenteritis in the week preceding their journey. Moreover, onset of disease in all cases was after day 1 of the journey, suggesting a common point source of infection in each group.

Norovirus outbreaks are common and most often spread by person-to-person transmission. Because of their high attack rates, short incubation period and the high stability of the infectious agent in the environment, norovirus outbreaks are difficult to control. In addition, the identification of a common food-borne source is complicated because person-to-person transmission rapidly takes over after initial introduction of the virus through food. In order to be able to identify whether the virus had initially been introduced through food, we made a distinction between early and late cases, as early cases were more likely to be infected through food. This allowed us to identify an association between being an early case and drinking juice served in containers.

Although not all associations with consumption of juice served in a self-tap container were statistically significant and the CI values included 1.0, we concluded that the results are nevertheless suggestive of an association, seeing as there was an RR of 3.02 for early cases in Coach B drinking juice at dinner on day 2, for whom the CI shifted more from the right than to the left, also after correcting for non-lineairity of this relationship. Non-significance here may be an issue of small numbers. The results when considering biological plausibility, although not significant, did show an increase in risk per day in Coach A and a decrease per day in Coach B while considering the number of meals at which juice was consumed each day. We consider this to support the hypothesis of environmental contamination which most probably is linked to the use of the handle of the juice container.

It is generally difficult to determine whether food was contaminated during production or during preparation by infected food handlers [11]. Also in our study, contamination of the juice itself cannot be ruled out. It is not known whether the juice was tested for the presence of viruses. Virus detection in food involved in outbreaks, however, is generally complicated due to the low dose of viruses in food which may be below the detection level. Moreover, leftovers are rarely available for analysis. In particular, viral detection in fruit can be hampered by the presence of acid juices inhibiting the

assay. Until validated assays are available, epidemiological evidence may assist in confirming a food-borne source [5]. However, the juice was served in a self-tap container during each meal buffet, making transmission through a contaminated environment possible. Also, our epidemiological evidence was indicative of transmission to the early cases through the contaminated juice container, with person-to-person transmission taking over for the later cases. Both groups were at highest risk on 9 February. It is possible that cleaning procedures in the hotel were different on days when tour groups changed due to tight time schedules, resulting in persisting environmental contamination. It is also possible that cleaning procedures after the arrival of the second coach reduced transmission and decreased the dose-response association of juice consumption for Coach B travellers. Unfortunately, we could not confirm this hypothesis since the report on the environmental investigation performed by the German public health office was not available for MPHS Rotterdam-Rijnmond.

Given the background prevalence of norovirus in the population and the presence of asymptomatic shedders, identifying norovirus as the causative agent of an outbreak of gastroenteritis is commonly based on a total of three samples, of which at least one should be positive for norovirus using RT-PCR [10]. In our study, only two samples in each group could be tested, but all were positive. Therefore, it is justified to assign the outbreak in both coaches to norovirus. Moreover, the strains detected were identical over a sequence length of 300 and 600 nt, strongly indicating that these outbreak strains were linked.

Often a case-definition of two or more episodes of diarrhoea and/or vomiting within 24 hours is used. We chose a case-definition of one or more episodes of diarrhoea and/or vomiting within 24 hours. As the 10 persons with only one such episode had also other symptoms which were strongly indicative of a norovirus infection, we consider our case definition justified.

Separating the early from the late cases appears to have been a successful method for distinguishing transmission modes, as described before [12]. An association with a food item was found in early cases, whereas person-to-person transmission or environmental transmission took over in the late cases. The late cases were probably predominantly infected through person-to-person spread of the virus in the coach on the way back to the Netherlands. Norovirus is known to spread easily from person to person in closed settings, as is seen for example on cruise ships [13]. After the passengers were back home in the Netherlands, the incidence fell rapidly and two days after departure from the hotel there were no new cases in either of the groups, suggesting that there was no exposure to a persisting common source or ill persons, and that providing the control guidelines contributed to limiting further spread.

Travelling has previously been described to be a risk factor for norovirus infection [14]. However, identification of the initial source in such outbreaks is difficult [15]. Outbreaks in travellers often involve several countries, which makes it difficult to collect all relevant data, a clear limitation of our study. In the Netherlands, cooperation between the various Public Health Services and the national Food and Consumer Product Safety Authority (VWA) is working well. If food-borne transmission is suspected, results of epidemiological and environmental investigations are jointly collected and shared. This is different from routine environmental control by the VWA. Different countries may have different surveillance systems, laws and regulations concerning privacy and sharing information. This interferes with international outbreak investigations and may profit from international guidelines and data sharing.

To our knowledge, this is the first time that consumption of juice served in containers is demonstrated to be a critical point in the hygiene procedure of hotels. Travel agencies and hotels should to be made aware that, once norovirus is introduced in a hotel setting, these containers need hygienic measures to limit further spread and prevent outbreaks in successive groups. Each outbreak investigation requires cooperation between actors in epidemiological, microbiological and environmental investigation, whether national or international. For a thorough investigation of outbreaks with international consequences, European guidance is needed regarding the collaboration of different authorities involved in cross-border outbreaks. In our opinion, the European Centre for Disease Prevention and Control is the body suited to provide such guidelines, in which it is important to address the potential lack of international comparability of laboratory data.

Acknowledgements

We would like to thank the local German Public Health Office (Gesundheitsamt) and the National Institute for Public Health and the Environment (RIVM) for their cooperation, Joukje Siebenga for the capsid sequences, the travel agency in providing the addresses of the travellers and the travellers themselves for their cooperation in returning the questionnaires.

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