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

Vol. 16 | Weekly issue 34 | 25 August 2011

Rapid communications

Ongoing outbreak of West Nile virus infection in humans, Greece, July to August 2011 2
by K Danis, A Papa, E Papanikolaou, G Dougas, I Terzaki, A Baka, G Vrioni, V Kapsimali, A Tsakris, A Kansouzidou, S Tsiodras, N Vakalis, S Bonovas, J Kremastinou

Surveillance and outbreak reports

Salmonellosis outbreak due to Salmonella Enteritidis phage type 14b resistant to nalidixic acid, Austria, September 2010 7
by L Hrivniaková, D Schmid, A Luckner-Hornischer, H Lassnig, C Kornschober, J Angermayer, F Allerberger

Research articles

Hepatitis A and hepatitis A virus/HIV coinfection in men who have sex with men, Warsaw, Poland, September 2008 to September 2009 14
by MM Dabrowska, K Nazzal, A Wiercinska-Drapalo

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Ongoing outbreak of West Nile virus infection in humans, Greece, July to August 2011

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Citation style for this article:
Danis K, Papa A, Papanikolaou E, Dougas G, Terzaki I, Baka A, Vrioni G, Kapsimali V, Tsakris A, Kansouzidou A, Tsiodras S, Vakalis N, Bonovas S, Kremastinou J. Ongoing outbreak of West Nile virus infection in humans, Greece, July to August 2011.
Euro Surveill. 2011;16(34):pii=19951. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19951

Article published on 25 August 2011

Between 16 July and 21 August 2011, 31 cases of West Nile neuroinvasive disease were reported from four regions in Greece. Of these, 17 occurred in districts that had not been affected in 2010. The reoccurrence of human cases in two consecutive years (following the large 2010 outbreak) and the spread of the virus in new areas suggest that West Nile virus is established in Greece, and its transmission may continue to occur in the future.

Since July 2011, an outbreak of West Nile virus (WNV) infection has been ongoing in regions in Greece that had already been affected in 2010, and in regions that had never reported human cases before. Here we present information on the extent of the ongoing outbreak and describe the geographical and temporal distribution of West Nile neuroinvasive disease (WNND) cases.

During 2010, Greece experienced the second largest outbreak of WNV infections in Europe since the one that had occurred in Romania in 1996 [1-3]. Overall, 262 cases of WNV infection in humans were notified mainly in northern Greece. In the Central Macedonia region all seven districts had reported cases and in the adjacent Thessalia region one of the four districts was affected. Among reported cases, 197 presented with WNND and 33 of these died, indicating a case fatality rate of 17% among WNND cases [1]. WNV lineage 2 sequences were obtained from three pools of *Culex* mosquitoes (strain Nea Santa-Greece-2010), from two blood donors and from wild birds [4-6]. This was the first time that WNV infection was documented in humans in Greece, although serosurveys in the early 1960's, 1980's and 2007 identified WNV antibodies in approximately 1% of the human population, suggesting that WNV, or a related flavivirus, was circulating in Greece [7-9].

Surveillance methods

As part of the general surveillance system, physicians in Greece are asked to notify the Hellenic Centre for Disease Control and Prevention (HCDCP) of all cases of WNV infection, using a slightly modified 2008 European Union (EU) case definition (the same as the one used in 2010) [2,10]. A confirmed case is defined as a person meeting any of the following clinical criteria: encephalitis, meningitis, fever without specific diagnosis and at least one of the four laboratory criteria: (i) isolation of WNV from blood or cerebrospinal fluid (CSF), (ii) detection of WNV nucleic acid in blood or CSF, (iii) WNV-specific antibody response (IgM) in CSF, and (iv) WNV IgM high titre, and detection of WNV IgG, and confirmation by neutralisation. A case is considered probable if the patient meets the above clinical criteria and a WNV-specific antibody response is demonstrated in his or her serum sample.

A standardised reporting form is used to collect information regarding the demographic characteristics, clinical manifestations, underlying chronic medical conditions, potential risk factors and laboratory results of the reported cases. Regular telephone inquiries to hospitals in the affected areas are conducted for case finding, follow-up and data validation. In addition, in-depth telephone interviews are conducted using a semi-structured questionnaire to obtain a detailed exposure history of all cases. Cases reported as encephalitis (including meningoencephalitis), meningitis, or acute flaccid paralysis, are classified as WNND cases. All other cases are considered non-neuroinvasive.

Laboratory methods

Serum and CSF specimens were tested for the presence of WNV-specific IgM and IgG antibodies using commercial ELISA kits (WNV IgM capture DxSelect and WNV IgG DxSelect, Focus Diagnostics Inc, Cypress, CA, USA). WNV positive specimens were also tested for the presence of other flaviviruses: tick-borne encephalitis virus (TBEV) and dengue virus (DENV).

Data analysis

Incidence rates were calculated using as denominator the 2007 mid-year population estimates of the Hellenic

Statistical Authority (HSA) [11]. Comparison of categorical variables was assessed using the chi-squared test. Risk ratios (RR) were calculated to compare incidence rates. The analysis was carried out using STATA version 10 software (Stata Corporation LP, Texas, USA). Data were mapped using the GNU R software (www.gnu.org/s/r/).

Results

By 21 August (week 33), 37 laboratory-diagnosed cases of WNV infection were reported to the HCDCP; 31 of these (24 confirmed and seven probable) presented as WNND cases and six of them (all probable) as non-neuroinvasive. This report focuses mainly on the 31 WNND cases, which were identified and reported more consistently, because of the disease severity. The overall incidence of WNND in the country was 0.28 cases per 100,000 population (Table).

For the 37 laboratory confirmed cases, 31 serum samples and 25 CSF specimens were available; for 19 patients both CSF and serum specimens were provided, while for six patients only CSF was available. WNV-specific IgM antibodies were detected in all 31 serum samples and in 24 CSF specimens, while WNV-specific IgG antibodies were detected in 15 serum and eight CSF specimens. In all 19 patients for whom both types of specimen were available, WNV-specific IgM antibodies were detected in both CSF and serum. As was the case in 2010, all specimens were negative for TBEV, while low level of cross-reactivity was seen in IgM with DENV [12]. None of the patients had been vaccinated for yellow fever.

The first case of WNND reported onset of symptoms on 16 July 2011 (week 28) (Figure 1). An increased number of cases was observed during weeks 30 and 32.

The median age of patients with WNND was 70 years (range 21–87) with the age-specific attack rate of WNND increasing significantly (p=0.009) with increasing age. The incidence in persons aged 70 years or older was approximately 23 times higher compared to that of individuals younger than 30 years (Table). Of all WNND cases, 19 were male. The place of residence of WNND cases is presented in Figure 2.

None of the cases reported travel abroad during the incubation period. The first cases occurred in northern Greece in Central Macedonia and Thessalia region, whereas approximately 10 days later, cases were reported for the first time from Eastern Attiki (in close proximity – approximately 43 km – to the metropolitan area of Athens). Overall, cases were distributed throughout nine of 54 Greek districts in four of 13 Greek regions. Of all WNND cases, 17 occurred in areas where cases had not been documented in 2010 (namely Karditsa and Trikala in Thessalia region, and Eastern Attiki and Viotia, in Central Greece). None of the cases had a history of recent blood transfusion or tissue/organ transplantation.

Table

Basic characteristics of reported cases of West Nile neuroinvasive disease, Greece, 16 July – 21 August 2011 (n=31)

Characteristic	Number of cases	Incidence rate[a] (per 100,000 population)	Risk Ratio (95% confidence interval)
Age group (years)			
<30	2	0.05	reference
30-49	2	0.06	1.10 (0.15–7.78)
50-59	3	0.21	3.91 (0.65–23.41)
60-69	5	0.42	7.77 (1.51–40.03)
≥70	19	1.26	23.30 (5.43–100.04)
Sex			
Female	12	0.21	reference
Male	19	0.34	1.61 (0.78–3.33)
District/prefecture (region) of residence			
Karditsa (Thessalia)	5	4.30	12.26 (3.29–45.66)
Eastern Attiki (Attiki)	10	2.48	7.05 (2.21–22.49)
Serres (Central Macedonia)	3	1.59	4.54 (1.02–20.27)
Larissa (Thessalia)	4	1.40	3.99 (1.00–15.95)
Imathia (Central Macedonia)	2	1.39	3.95 (0.72–21.58)
Viotia (Sterea Ellada)	1	0.80	2.27 (0.25–20.32)
Trikala (Thessalia)	1	0.77	2.18 (0.24–19.50)
Pella (Central Macedonia)	1	0.69	1.96 (0.22–17.57)
Thessaloniki (Central Macedonia)	4	0.35	reference
Total	**31**	**0.28**	

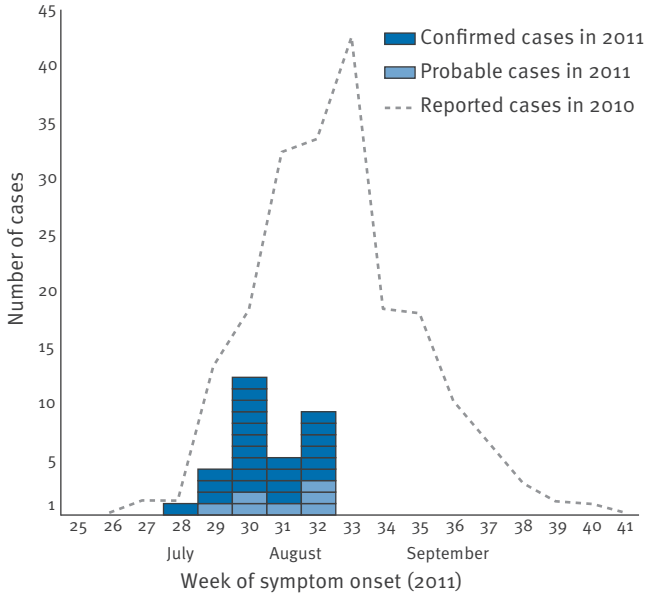
[a] Incidence rates were calculated using as denominator the 2007 mid-year population estimates of the respective groups available from the Hellenic Statistical Authority.

Of all WNND cases, 22 presented with meningoencephalitis and nine with meningitis alone. Two patients presented with a combination of meningoencephalitis and acute flaccid paralysis. Information on underlying chronic medical conditions was available for 23 of the WNND cases: 17 had at least one underlying disease, with the most common being hypertension (n=11), diabetes mellitus (n=7) and coronary artery disease (n=6). All WNND cases were hospitalised and six were admitted to an intensive care unit (ICU). As of 21 August 2011, one case (aged over 70 years) who had several underlying conditions, had a fatal outcome.

There were also six non-WNND cases reported, who probably represent a very small fraction of all non-WNND, as mild WNV cases are less likely to seek medical care and be identified. In depth interviews were conducted with all of them. The median age of the reported non-WNND cases was 44 years (range 10-78) and was significantly different (p=0.009) from that of non-WNND ones (median age 70 years; range 21-87). Of those, five were hospitalised but none in an ICU. With regard to specific symptoms, fever was reported by all of them, followed by headache (n=3), rash (n=2), weakness (n=2) and nausea/vomiting or diarrhoea (n=1).

Figure 1

Reported cases of West Nile neuroinvasive disease by week of symptom onset, Greece, 16 July – 21 August 2011 (n=31)

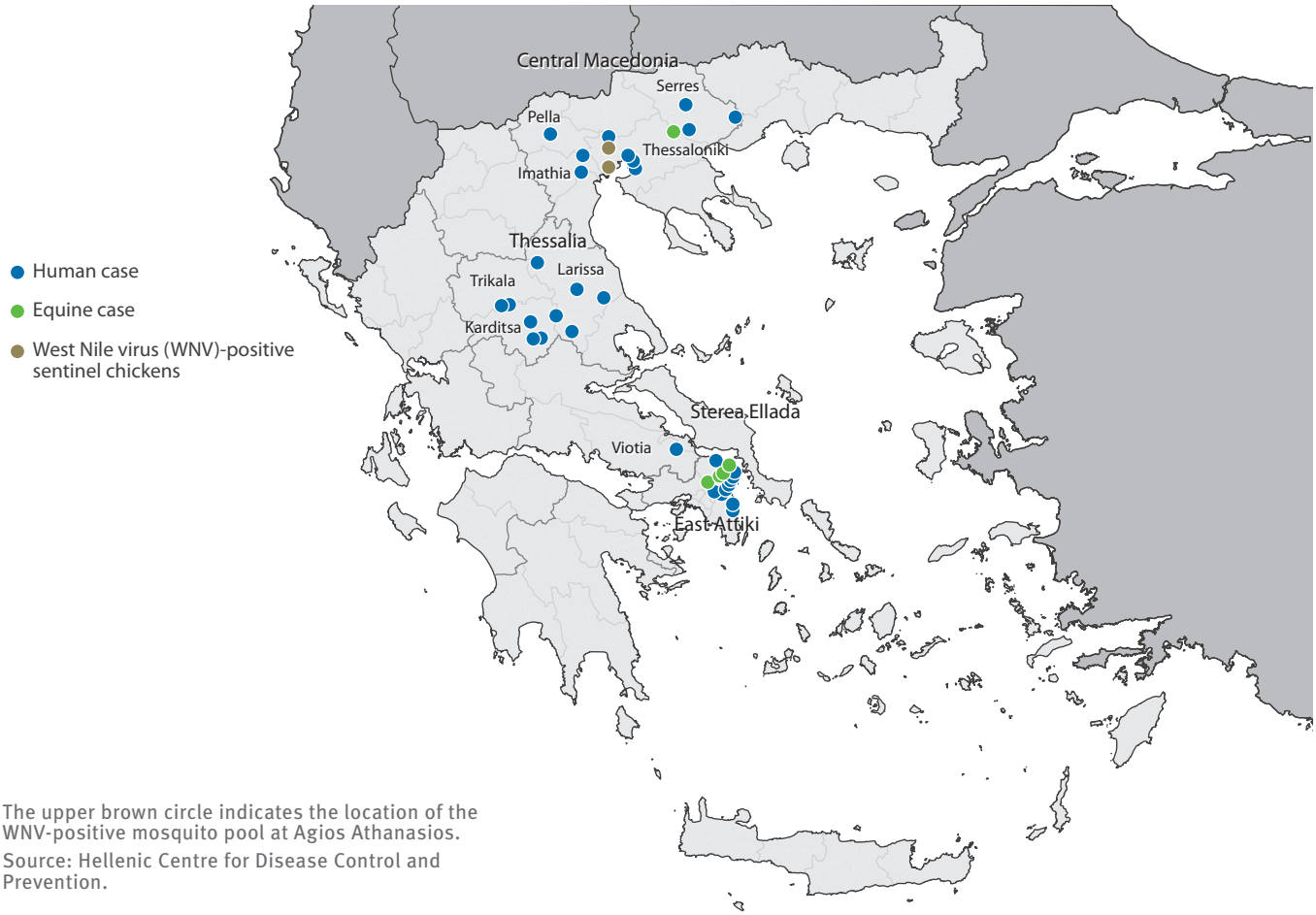


As this is an ongoing outbreak, the number of 2011 cases corresponding to previous weeks may increase as more cases are confirmed retrospectively.

Source: Hellenic Centre for Disease Control and Prevention.

Figure 2

Place of residence of reported cases of West Nile neuroinvasive disease, Greece, 16 July – 21 August 2011 (n=31)



The upper brown circle indicates the location of the WNV-positive mosquito pool at Agios Athanasios.

Source: Hellenic Centre for Disease Control and Prevention.

Discussion and conclusions

Following the large WNV outbreak in 2010, WNV human infections are notified in Greece for a second consecutive year. As of 21 August, 31 WNND were reported from four regions in Greece. Human cases of WNV infection were also detected in other European and Mediterranean countries during this season (as of 18 August, two cases had been reported in Romania, 21 in the Russian Federation, two in Albania, and five in Israel) [13].

The 2010 outbreak in Greece was mainly localised in Central Macedonia. Although cases seem to reoccur in the same districts as in 2010, a new geographic pattern of WNV spread is being observed in 2011. Following the intense WNV amplification and transmission in Central Macedonia in the previous year, the virus seems to disperse southward to the newly affected areas of Thessalia region (Karditsa) and further south to East Attiki (approximately 500 km from Central Macedonia), in proximity to the metropolitan area of Athens. Similar dispersal patterns have been observed in California, which has a similar climate to Greece, where WNV was introduced in 2003 and quickly spread throughout the state [14,15].

The temporal distribution of cases shows that the first human cases in 2011 occurred approximately two weeks later compared to 2010. Comparing the magnitude of the current outbreak in Central Macedonia to the outbreak in the previous year, the current one remains lower to date, suggesting decreased or delayed WNV transmission in humans. Due to the high visibility of the 2010 outbreak and the subsequent raised awareness to the infection among physicians, it is unlikely to be due to delayed recognition of the disease or under-reporting. However, as this is an ongoing outbreak, the number of 2011 cases corresponding to previous weeks may increase as more cases are confirmed retrospectively.

In early May and June 2011, WNV transmission was detected by seroconversions of sentinel chickens and domestic pigeons in Central Macedonia [15]. Lineage 2 WNV sequences were obtained from one pool of *Culex pipiens* mosquitoes trapped in the city of Agios Athanasios (40º43'0.59''N, 22º44'7.04''E) west of Thessaloniki on 23 June 2011. This strain showed the highest homology (99.4%) to the Nea Santa/Greece/2010 WNV strain detected in *Culex pipiens* in 2010. An identical strain was also detected in seroconverted sentinel chickens in the same city on 13 July 2011 [15]. Genetic characterisation of WNV strain(s) of 2011 circulating in other areas will elucidate whether it is an identical strain or a newly introduced one.

Regarding equidae, five horses from East Attiki presented with clinical WNV disease manifestation, as well as one horse from Serres (Central Macedonia) with antibodies against WNV that demonstrated a recent infection (IgM positive) (Figure 2) [16]. The first two cases in horses were identified before human cases had been reported in Attiki and thus functioned as an early warning signal.

Following the large 2010 WNV infection outbreak, a number of public health measures were implemented in 2011:

- guidelines for healthcare professionals for the recognition, management and diagnosis of encephalitis and WNV infection in order to improve their awareness regarding the disease;
- enhanced surveillance of encephalitis and WNV infection in humans;
- a project on mosquito mapping across the country;
- a project on mosquito surveillance;
- a seroprevalence study of WNV infection among humans in the epicentre of the 2010 WNV infection outbreak;
- a WNV seroepidemiological study in domestic pigeons and poultry;
- multi-sectoral collaboration and exchange of information between human health, veterinary health and entomological sectors;
- guidance for blood and blood product safety according to the EU directives;
- communication and health promotion activities encouraging personal protection against mosquito bites in the general population;
- vector control activities.

In conclusion, the reoccurrence of human WNV infection cases in two consecutive years and the spread of the virus in newly affected areas, suggest that WNV is established in Greece and transmission may continue in the future. Intensified vector mosquito control programmes, along with ongoing public health education, integrated human and animal WNV surveillance to monitor the spread of the virus and implementation of blood transfusion measures are necessary to prevent transmission and control the disease.

Updates on reported WNV cases in Greece are published in the Weekly Surveillance Reports available in English on the HCDCP website [17].

Acknowledgments

We would like to thank all hospital physicians and local public health authorities who contributed to the surveillance of WNV infections in Greece; the personnel of the University and Hospital laboratories for technical support; and Athanasiou M, Detsis M, Kallimani A, Sapounas S, Balaska A, Ntakou A, for their support in surveillance activities.

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Salmonellosis outbreak due to *Salmonella* Enteritidis phage type 14b resistant to nalidixic acid, Austria, September 2010

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Citation style for this article:
Hrivniaková L, Schmid D, Luckner-Hornischer A, Lassnig H, Kornschober C, Angermayer J, Allerberger F. Salmonellosis outbreak due to Salmonella Enteritidis phage type 14b resistant to nalidixic acid, Austria, September 2010.
Euro Surveill. 2011;16(34):pii=19952. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19952

Article published on 25 August 2011

We report on a salmonellosis-outbreak due to *Salmonella* Enteritidis phage type 14b resistant to nalidixic acid (*S.* Enteritidis PT14b Nx) among residents and employees of a student residence in Austria, September 2010. The outbreak was described and analysed by a retrospective cohort study, and microbiological environmental investigations were conducted to identify the outbreak source(s) and the reservoir of the outbreak strain. A total of 66 persons fulfilled the outbreak case definition including 14 laboratory-confirmed cases. Food specific cohort-analyses by day revealed that consumption of potato salad (RR: 1.65, 95%CI: 1.35–2.01, p=0.001) and a cheese-sausage cold plate (RR: 2.24, 95%CI: 1.29–3.88, p=0.002) on 14 September was associated with being an outbreak case. We hypothesised that cross-contamination with *S.* Enteritidis PT14b Nx positive eggs had occurred during preparation of the potato salad and cold plate as a result of preparing in parallel egg-containing breaded cutlets on 14 September. A traced laying hen holding in eastern Austria was identified as the sole source of the consumable eggs in the student residence. By applying the legally mandated sampling method for epidemiological-related laying hen farms (one pooled dust sample à 150g, two paired boot swabs cultured separately), the outbreak strain could not be detected. Our findings, that legally required sampling methods for laying hen farms failed to detect the causative pathogen in a laying hen holding, despite an epidemiological link, underline the request stated by the European Food Safety Authority Panel on Biological Hazards for a more sensitive sampling plan in epidemiologically-associated laying hen flocks.

Introduction

In the European Union (EU), food-borne outbreaks are mandatorily reported since 2003 [1]. Since then, between 30% and 60% of the reported outbreaks have been caused by *Salmonella* [2]. In Austria, among the 1,255 *Salmonella* outbreaks reported within the past five years, the most frequent serovar was *Salmonella* Enteritidis, accounting for more than 82% (unpublished data).

Eggs from *Salmonella*-positive laying hen flocks and products made from such eggs were the most frequently associated food vehicles [2].

Before 2001, *S.* Enteritidis phage type (PT) 14b was considered a rare causative agent of human salmonellosis in the EU [3]. In 2001, Norway, Sweden and Finland reported increased numbers of cases of infection with *S.* Enteritidis PT14b in patients who had travelled to Greece [3]. In 2002, an outbreak with continuing exposure to a source of infection, associated with eating bakery products, was observed in the United Kingdom and was caused by *S.* Enteritidis PT14b susceptible to nalidixic acid [4]. In Austria, from 2001 to 2003, only 1.8% of the total 21,247 *S.* Enteritidis cases registered, were of *S.* Enteritidis PT14b and all were susceptible to nalidixic acid. In these cases, there was no history of travel.

In 2009, an upsurge in the number of non-travel associated cases of infection with *S.* Enteritidis PT14b resistant to nalidixic acid (*S.* Enteritidis PT14b Nx) was observed in the United Kingdom and linked to the consumption of egg-containing food [5]. In Austria, cases of infection with *S.* Enteritidis PT14b Nx were first reported in 2005 and accounted for 0.3% of the 4,669 registered *S.* Enteritidis cases. From 2006 onwards the proportion of *S.* Enteritidis PT14b Nx among *S.* Enteritidis cases increased continuously: 0.3% in 2006, 0.9% in 2007 and 1.5% in 2008. In 2009, none of the isolates from 20 cases of *S.* Enteritidis PT14b, among the registered 1,829 cases of *S.* Enteritidis, were resistant to nalidixic acid, according to the data of the Austrian reference laboratory for *Salmonella*.

We report on the first documented food-borne outbreak due to *S.* Enteritidis PT14b Nx, which occurred in September 2010 in Austria, and discuss different

environmental sampling methods with respect to the sensitivity of detecting the outbreak strain in epidemiologically-linked poultry flocks.

Outbreak description

At the end of September 2010, the Austrian Agency for Health and Food Safety (AGES) was informed by the Austrian reference laboratory for *Salmonella* of a cluster of 14 cases of gastroenteritis due to infection with *S.* Enteritidis PT14b Nx in a western province of Austria. These cases had occurred in a student residence after its re-opening, following the summer break, on 12 September. Another 30 cases of gastroenteritis among the residents of the student residence were reported by the public health authority by 30 September 2010. The student residence hosted 142 male students and 19 staff, which included five kitchen workers, seven tutors and seven administrative staff.

In the previous five years, from 2005 to 2009, a total of seven cases of 40 *S.* Enteritidis PT14b that had been registered in this western province showed antimicrobial resistance to nalidixic acid, according to the data of the Austrian reference laboratory for *Salmonella*.

AGES was mandated by the competent public health authority to investigate the student residence outbreak on 30 September. The aim of the investigation was to describe the outbreak epidemiologically to identify the outbreak source(s) and the reservoir of the causative pathogen in order to set appropriate control and preventive measures.

Case definition

The following outbreak case definition was applied: A probable outbreak case was defined as a person who (i) was a resident or working as kitchen staff or tutor in the particular student residence from 12 September onwards, and (ii) fell sick with symptoms of gastroenteritis (at least three loose stools per day or vomiting) on 13 September at the earliest. A confirmed outbreak case was defined as a person who fulfilled criteria (i) and (ii) and tested positive for *S.* Enteritidis PT14b Nx. In personal interviews, cases were asked about demographics, disease onset, symptoms, hospitalisation and duration of disease, and whether they had stool samples handed in for testing.

A total of 66 persons fulfilled the outbreak case definition including 52 probable (only student-cases) and 14 confirmed outbreak cases (involving 13 student-cases and one tutor-case). The duration of diarrhoea ranged from one to 10 days with a mean of 4.4 days. The median age of the cases was 16.8 years (range: 14.1–21.1); cases were all male and diarrhoea was the dominant symptom (62/66, 94%).

Outbreak characteristics

The outbreak occurred from 14 September to 21 September and peaked with 29 cases on 16 September. The pattern indicated a point source active on 14 September followed by a continuous common source (Figure).

Methods

Retrospective cohort study

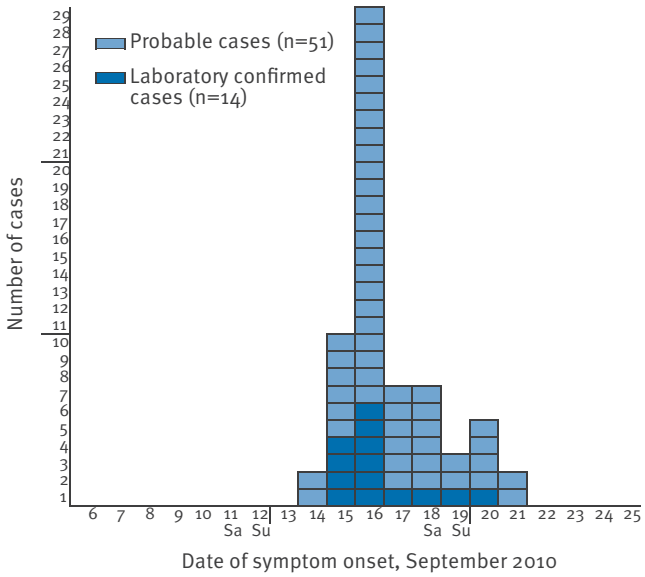
It was hypothesised that food offered by the residence kitchen was the most likely source of the outbreak. An analytical epidemiological investigation was performed using a retrospective cohort study in order to identify the food item(s) most likely associated with the risk of infection with the outbreak strain *S.* Enteritidis PT14b Nx and to generate a hypothesis on the reservoirs of the outbreak strain.

Cohort of interest and food exposure history

The student residence hosted students aged between 14 and 18 years from Sunday evening until Friday midday. According to information provided by the local public health authorities, the cohort of interest comprised 161 persons with 142 student residents and 19 employees (five kitchen workers, seven tutors and seven administrative staff), present from 12 to 17 September. Meals were offered three times a day, made in the kitchen, served and consumed in the residence refectory. The menus for 12 September, evening until 17 September, lunch (no dinner was offered on 17 September), were provided by the kitchen chef. A continental breakfast was served daily and included muesli containing wheat, oats and corn eaten with milk or yogurt. On Sunday evening, when the student residence kitchen re-opened after the summer break, a ham and cheese toast was offered for dinner, on Monday spaghetti with salad were served for lunch and frankfurters with baked roll for dinner, on

Figure

Outbreak cases of *Salmonella* Enteritidis phage type 14b resistant to nalidixic acid, by day of symptom onset, Austria, 14–21 September 2010 (n=66)



Date of symptom onset is unknown for one outbreak case.

Tuesday breaded cutlets (i.e. traditional Austrian dish "Wiener Schnitzel") and potato salad were offered for lunch and a cold plate for dinner, on Wednesday pizza with salad for lunch and noodles with ham for dinner, on Thursday fish sticks with vegetable rice for lunch and fried chicken with mixed salad for dinner, and on Friday, pancakes with cheese or with jam were served for lunch.

Data collection and analysis

Data on food exposure for the days from 12 to 17 September were ascertained by a self-administered questionnaire. Food-specific attack rates (AR) and relative risks (RR) were calculated for a total of 27 dishes and food items regardless of the day on which a specific food item had been served. The data were entered into Epi Info version 3.5.1 and STATA version 11 was used for univariate and stratified analyses. Differences in food-specific AR between exposed and unexposed groups were tested by chi-square or Fisher's exact test yielding the RR with a 95% confidence interval. In a second approach, food-specific cohort analyses were performed for each relevant day (12 September to 17 September). A specific study cohort was defined for each day including disease free members only, i.e. outbreak cases occurring the days prior to or on the day under study were excluded from the respective day-specific study cohort. A diseased person was defined as a member of the day-specific study cohort who had fallen sick with symptoms of gastroenteritis within three days following exposure to the food item of the specific day under study (considering a maximum incubation period of 72 hours). Exposure to cereals was defined as consumption of muesli at breakfast on any day from 13 until 19 September, because the day-specific consumption of muesli could not be recalled by the cohort members.

Microbiological and environmental investigation

As initiated by the outbreak investigators on 30 September, stool samples from five kitchen workers and six tutors, who had all remained asymptomatic throughout the outbreak, were tested for *Salmonella*. Isolates were serotyped according to the Kauffmann-White scheme, and phage-typed as described elsewhere [6,7]. Samples obtained from the kitchen environment were tested as described previously [8].
As part of the environmental investigation, the laying hen holding identified as the source of the eggs used in the relevant period by the student residence was sampled for *Salmonella* testing, by one sample of pooled dust à 150 g and two paired boot swabs per flock (cultured separately). This method was according to the official sampling conducted once a year, which is done in addition to the 15 week sampling within the regulatory monitoring program [9]. Microbiological workup of these environmental samples was performed as described elsewhere [10]. In addition to microbiological testing within the outbreak investigation, we reviewed the results of *Salmonella* testing within the regulatory monitoring program.

No food samples were available for testing when the outbreak investigation was performed.

Multiple-locus variable number tandem repeat analyses and pulsed-field gel electrophoresis
Pulsed-field gel electrophoresis (PFGE) by use of the restriction enzyme *Xba*I and multiple-locus variable number tandem repeat analyses (MLVA) were performed with the human isolates of *S.* Enteritidis PT14b Nx obtained from student-cases and with the environmental isolates [11,12].

Results

Retrospective cohort study

Completed questionnaires from 144 of 161 persons of the cohort of interest were provided (response rate 90%) giving a study cohort of 141 students (including 65 student-cases), two tutors (one tutor case) and one kitchen worker. The food-specific cohort-analyses yielded consumption of breaded cutlet (RR: 3.97, 95%CI: 1.37–11.50, p<0.0001), potato salad (RR: 3.58, 95%CI: 1.43–8.95, p=0.000), spaghetti (RR: 2.31, 95%CI: 1.05–5.08, p=0.010), frankfurters (RR: 2.22, 95%CI: 1.23–4.01, p=0.001), baked roll (RR: 2.12, 95%CI: 1.21–3.70, p=0.002), meat and cheese cold plate (RR: 2.06, 95%CI: 1.34–3.19, p=0.000) and bread slices (RR: 2.00, 95%CI: 1.30–3.09, p=0.08) as factors significantly associated with the infection risk. Of these seven dishes, the following food items were re-identified as risk associated by the food-specific analyses by day: cold plate (RR: 2.24, 95%CI: 1.29–3.88, p=0.002), bread slices (RR: 2.17, 95%CI: 1.25–3.76, p=0.002), breaded cutlet (RR: 1.69, 95%CI: 1.41–2.02, p=0.001) and potato salad (RR: 1.65, 95%CI: 1.35–2.01, p=0.001) served at lunch on 14 September, served at dinner on 14 September (Table 1). After stratifying the effect of breaded cutlet and bread consumption by the exposure status to potato salad, and the effect of bread consumption by the exposure status to the cold plate, eating breaded cutlet and eating bread slices became insignificant.

A total of 52 (96%) of the 54 outbreak cases having occurred from the evening of 14 September, until the morning of 18 September had consumed the potato salad at lunch on 14 September, and 40 (77%) of the 52 outbreak cases having occurred from 15 to 18 of September, ate the cold plate at dinner on 14 September.

Eating muesli at any day at breakfast from 13 until 17 September revealed a RR of being a case of 1.51 (95%CI: 1.01–2.28, p=0.037).

Microbiological and environmental investigation

One of the five kitchen workers and one of five tutors without symptoms of gastroenteritis tested positive for

S. Enteritidis PT14b Nx. All 20 environmental samples taken from the kitchen tested negative for *Salmonella*.

A laying hen holding in eastern Austria was identified as the sole source of consumable eggs for the residence. This laying hen holding comprised four business premises (A, B, C and D) dispersed across three districts. Premise C was identified to have provided eggs to a local retailer in the outbreak-province, September week 1 (on 7 September), from which the residence manager subsequently purchased the eggs for September week 2, for the re-opening after the summer break. The review of the results of the regulatory operator monitoring revealed that in premise C, one of the 12 flocks (premise C flock I, involving 18,000 laying hens) had tested positive for *S.* Infantis in June and positive for *S.* Enteritidis PT8 on 14 September. The marketing ban applied on 28 September had been continued, after *S.* Enteritidis PT8 was also found among 4,000 eggs (1/99 pools of 40 eggs was positive) from premise C flock I. A neighbouring flock, premise C flock II, consisting of 19,500 laying hens - tested positive for *S.* Enteritidis PT19 at the beginning of December (Table 2).

As premise C was assumed to be the most likely source of the eggs for the student residence, in the relevant time period, and without knowing the egg-producing flock(s), the two flocks that had already tested positive for *Salmonella* in the previous months (premise C flocks I, II) were sampled and re-tested for *Salmonella* within the outbreak investigation end of December. The sampling method included a single sample of pooled dust and two pairs of boot swabs (cultured separately) from each flock. In premise C flock I *S.* Enteritidis PT8 was detected in two samples (one dust sample, one boot swab) and *S.* Enteritidis PT19 in one sample (one boot swab) of five (one dust sample, four boot swabs). In none of these five environmental samples was *S.* Enteritis PT14b detected. In premise C flock II, one

Table 1

Day-specific cohort analysis by food exposure, *Salmonella* outbreak, Austria, 14–21 September 2010

Date	Day-specific cohort	Meal	Food items	Food exposed			Food unexposed			Univariable analyses		
				Cases	Total Number of exposed cohort members	Attack rate, as %	Cases	Total Number of unexposed cohort members	Attack rate, as %	Relative risk	95% C.I.	p-value
Sep 12	144	Dinner	Ham and cheese toast	6	71	8	6	73	8	1.03	0.35–3.04	0.960
Sep 13	144	Lunch	Soup	8	22	36	33	122	27	1.34	0.72–2.51	0.373
			Spaghetti	38	122	31	3	22	14	2.28	0.77–6.75	0.094
			Green Salad	20	64	31	21	80	26	1.19	0.71–2.00	0.509
		Dinner	Frankfurters, mustard	34	108	31	7	36	19	1.62	0.79–3.33	0.166
			Baked roll	33	106	31	8	38	21	1.48	0.75–2.91	0.237
Sep 14	144	Lunch	Soup	7	16	44	47	128	37	1.12	0.71–1.76	0.784
			Breaded cutlet	53	122	43	1	22	4	1.69	1.41–2.02	0.001
			Potato salad	52	118	44	2	26	8	1.65	1.35–2.01	0.001
	142	Dinner	Cold plate	40	85	47	12	57	21	2.24	1.29–3.88	0.002
			Bread slices	40	86	47	12	56	21	2.17	1.25–3.76	0.002
Sep 15	132	Lunch	Soup	4	15	27	39	117	33	0.80	0.33–1.92	0.604
			Pizza	43	125	34	0	7	0	NA	NA	0.059
			Green Salad	19	65	29	24	67	36	0.82	0.50–1.34	0.419
		Dinner	Noodles with ham	35	107	33	8	25	32	1.02	0.54–1.92	0.946
			Salad	16	49	33	27	83	33	1.00	0.60–1.67	0.988
Sep 16	103	Lunch	Soup	3	17	18	14	86	16	1.08	0.35–3.37	0.890
			Fish sticks	11	75	15	6	28	21	0.68	0.28–1.68	0.411
			Dip	10	61	16	7	42	17	0.98	0.41–2.38	0.971
			Vegetable rice	12	64	19	5	39	13	1.46	0.56–3.84	0.432
			Green Salad	4	44	9	13	59	22	0.41	0.14–1.18	0.080
		Dinner	Fried chicken	17	88	19	0	15	0	NA	NA	0.062
			Bread	14	64	22	3	39	8	2.84	0.87–9.27	0.060
			Mixed salad	11	56	20	6	47	13	1.54	0.62–3.85	0.349
Sep 17	96	Lunch	Soup	3	17	18	12	79	15	1.16	0.37–3.67	0.800
			Pancake	11	53	21	4	43	9	2.23	0.76–6.51	0.124
			Milk	3	15	20	12	81	15	1.35	0.43–4.22	0.611

NA: not applicable.

dust sample tested positive for *S.* Enteritidis PT8, and one of the four boot swabs for *S.* Enteritidis PT8 and PT19, but negative for *S.* Enteritidis PT14b (Table 2). A marketing ban was imposed on the eggs from premise C flock II at the end of December 2010. The other 10 flocks of premise C were not tested within the outbreak investigation.

Multiple-locus variable number tandem repeat analyses typing results

Six *S.* Enteritidis PT14b isolates, arbitrarily chosen from the 13 laboratory-confirmed student-outbreak cases, were further characterised by PFGE and MLVA and found to be indistinguishable from each other (MLVA-pattern: 9–6–5), but different from the four *S.* Enteritidis PT8 and the two PT19 isolates found in the environment of the premise C-flock I and flock II (PFGE patterns not shown; MLVA-results listed in Table 2). All environmental isolates were susceptible to nalidixic acid.

Discussion

We report the first food-borne outbreak due to *S.* Enteritidis PT14b Nx documented in Austria, which occurred in 2010, after no case of *S.* Enteritidis PT14b Nx had been identified in 2009. The food-specific cohort analyses revealed eight dishes as significantly associated with infection risk. Of these, cooked frankfurters, baked rolls or refined spaghetti appeared non-plausible as sources of infection considering the mode of their preparation. After an analysis of the food-specific AR by day, potato salad and cheese-sausage cold plate remained the most plausible outbreak sources. 96 percent of the cases that occurred from the evening of 14 September, until the morning of 18 September, could be explained by eating potato salad served at lunch on 14 September and consumption of foods on the cold plate, served at dinner on 14 September explained 77 percent of cases from 15 to 18 September. The outbreak pattern indicates a point source outbreak with 14 September as the most likely day on which common exposure occurred.

With respect to the maximum incubation period of approximately three days for *S.* Enteritidis, the 11 cases which occurred from the evening of 18 September until 21 September cannot be explained by consumption of the potato salad or cold plate. Even though the risk analysis of eating muesli any day at breakfast during the week of 13 until 17 September revealed only a weak association with the infection risk indicated by a RR of 1.51 (95%CI: 1.01–2.28, p=0.037), at least 50 of the 66 (76%) outbreak cases could also be explained by this food item. The muesli being a possible continuous common source of *Salmonella* in this outbreak is also biologically plausible, as the left-over muesli on the buffet table were returned into the same storage bowl to be served the next day. If contamination had occurred in the kitchen, where the muesli was arranged, then *Salmonella* growth would be enabled in the muesli left in un-refrigerated storage as described in documented salmonellosis outbreaks linked to breakfast cereals [13,14]. There was no muesli left for microbiological testing.

We hypothesised that eggs were the most likely source of contamination with *S.* Enteritidis PT14b Nx of the potato salad and cold plate based on the knowledge gained from the investigation of a large increase in non-travel-associated cases of *S.* Enteritidis PT14b Nx observed in 2009, in England and Wales. This upsurge in non-travel associated cases included at least 16 outbreaks and was epidemiologically and microbiologically traced back to imported Spanish eggs [5]. Breaded cutlet a traditional Viennese dish is dunked

Table 2

Positive *Salmonella* test results at the epidemiologically-linked laying hen holding premise C, within the regulatory operator monitoring and within the *Salmonella* outbreak investigations, as well as control measures, Austria, June 2010–January 2011

Flock	Date of sampling	Type of sample	Serovar Phage Type	MLVA pattern	Measures
Testing within regularly operator monitoring					
Flock I[a]	29 Jun 2010	Dust sample	*S.* Infantis	nd	Not mandatory[b]
Flock I[a]	14 Sep 2010	Boot swab	*S.* Enteritidis PT8	PT8: 10–5–7	From 28 Sep 2010 marketing ban on fresh eggs
Flock I[a]	29 Sep 2010	eggs (4,000)	*S.* Enteritidis PT8	PT8: 10–5–7	Culling of the flock in Oct 2010
Flock II	06 Dec 2010	Boot swab	*S.* Enteritidis PT19	nd	Measures taken in concert with the outbreak investigation
Testing within the outbreak investigation					
Flock I[a]	20 Dec 2010	Dust sample	*S.* Enteritidis PT8	PT8: 10–5–7	From 20 Dec 2010 marketing ban on fresh eggs; culling of the flocks in Jan 2011
Flock I[a]	20 Dec 2010	Boot swab	*S.* Enteritidis PT8, PT19	PT19: 9–5–7	
Flock II	20 Dec 2010	Dust sample	*S.* Enteritidis PT8	PT8: 9–5–7	
Flock II	20 Dec 2010	Boot swab	*S.* Enteritidis PT8, PT19	PT8: 9–5–7 PT19: 10–5–7	

MLVA: multiple-locus variable number tandem repeat analyses; nd: not done; PT: Phage type.

[a] Hens in Flock I were replaced after culling in Oct 2010.

[b] Mandated control measures only for *S.* Enteritidis and *S.* Typhimurium in Austria.

in flour, raw eggs and breadcrumb before being fried. The breaded cutlet was offered at lunch the day on which the potato salad and the cold plate were served. It is possible that cross-contamination occurred with *S.* Enteritidis PT14b Nx-positive eggs during pealing and cutting the boiled potatoes for the salad before adding the vinegar marinade, and during arranging the cold plate as a result of the parallel preparation of the breaded cutlets. Potato salad accompanying breaded cutlets is a well documented dish associated with outbreaks of salmonellosis in Austria [15].

A number of measures were taken to control and prevent further spreading of the outbreak. In the student residence, the kitchen was thoroughly cleaned and hand washing was reinforced on order of the school superior. Based on the assumption that eggs were the most likely source of contamination for the two risk-associated dishes, the egg-producer was traced. A large laying hen holding including four premises dispersed across two districts in eastern Austria turned out to have been the single egg-source for the student residence prior to the outbreak. The eggs consumed in the residence in September week 2 originated from the largest premise including 12 flocks. The two flocks of this premise, which had already tested positive for *S.* Enteritidis PT8 in September and for PT19 at the beginning of December, 2010 within the regulatory 15 week operator monitoring program, were re-investigated within the outbreak investigation end of December 2010 by testing a sample of 150 g pooled dust and two pairs of boot swabs per flock. This legally mandatory sampling method for epidemiologically-associated laying hen farms is in accordance to the sampling method employed once a year within the regulatory monitoring program, in addition to the 15 week sampling [9]. Even though this yearly sampling scheme is more sensitive compared to the 15 week sampling scheme (two paired boot swabs only cultured as one sample) [16], the outbreak strain *S.* Enteritidis PT14b Nx could not be detected. The isolates of *S.* Enteritidis PT8 and PT19 found in the two tested flocks of the related laying hen farm were susceptible to nalidixic acid and also distinguishable from the outbreak strain by the PFGE and MLVA pattern.

Although environmental sampling is usually the most effective way to detect *Salmonella* in poultry flocks [16,17], there are several reasons for failing to detect the outbreak strain in environmental samples from laying hen flocks despite strong epidemiological indications for causal association with a salmonellosis outbreak: (i) sampling of the wrong flock(s), (ii) sampling of the flock(s) that produced the contaminated eggs for the outbreak but at a time when the flock is no longer shedding *Salmonella*, considering that most hens stop shedding the bacteria after approximately three weeks [18,19] or (iii) a too low degree of shedding in vaccinated flocks resulting in a low within-flock prevalence, which is below the detection level of either the microbiological method or the sampling procedure [20,21]. In the outbreak described here, the two flocks of the associated laying hen premise (premise C flocks I and II), which were tested within the outbreak investigation, may not have produced the eggs for the residence before the outbreak took place. But the other ten flocks of premise C, which were not tested within the outbreak investigation, may have produced the infected eggs consumed in the residence. Another reason for not detecting the outbreak strain *S.* Enteritidis PT14b Nx in the two flocks tested, may be that the hens had already stopped shedding or the number of collected samples may be too low to detect *S.* Enteritidis PT14 Nx besides the persisting strain *S.* Enteritidis PT8 and PT19. During testing of samples, competing organisms are a limiting factor in detection. Low numbers of *Salmonella* organisms, e.g. less than 10 colony forming unit /g, can be especially difficult to identify against an overwhelming background of other dominant *Salmonella* strains [22,23]. These may include dominant phage types of *S.* Enteritidis such as PT4 and PT8 or some live vaccine strains.

Our findings that microbiological tests failed to isolate the outbreak causative pathogen from a laying hen holding despite an epidemiological link to human salmonellosis cases could suggest that the currently mandated sampling plan is not sensitive enough. It is important to recommend a more sensitive sampling plan for epidemiologically-associated laying hen flocks compared to the sampling method employed in the regulatory operator monitoring program [24], given the mandatory vaccination of laying hens against *S.* Enteritidis in Austria since 2008 [9]. Vaccination reduces the risk of *Salmonella*-positive eggs, but also hampers the likelihood of detecting infected flocks as a result of lowering of the within-flock prevalence and the number of organisms shed in faeces [25-28]. Arnold et al. [16] compared three different sampling methods with respect to the sensitivity for detecting infected flocks: method I involving 10 dust and 10 faecal samples per flock, method II according to the EU baseline study on the prevalence of *Salmonella* in laying flocks, which includes two dust samples à 250 ml and five paired boot swabs (each boot swab pair represents one fifth of the flock) [29] and method III involving single samples of pooled faeces and dust (i.e. 2 specimens per flock), which is according to the monitoring method in the National Control Program across Europe. Method I was most sensitive with a 98% power to detect 0.1% prevalence. These findings indicate that culturing several samples as indicated in method I, is more sensitive in detecting infected flocks than testing one single sample representing a large proportion of the flock. As a result of the present study, the Austrian AGES advocates the stringent methodology employed in the EU baseline survey (two dust samples and five paired boot swabs per flock) as sampling procedure for an outbreak-related laying hen farm including all flocks of a laying hen farm. However, in Austria the legally mandated sampling method for epidemiologically traced laying hen farms

involves only one pooled dust sample and two paired boot swabs per flocks.

With respect to the described risk factors for failure to detect an outbreak strain, a suspected laying hen holding should not be excluded as potential reservoir of a food-borne outbreak when there is reasonable epidemiological evidence for it.

Guidance from EFSA on the appropriate sampling method for epidemiologically traced laying hen flocks in food-borne outbreaks, which guarantees sufficient sensitivity for detecting infected flocks, is highly required to improve the quality of investigation of food-borne outbreaks in Member States.

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Hepatitis A and hepatitis A virus/HIV coinfection in men who have sex with men, Warsaw, Poland, September 2008 to September 2009

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Citation style for this article:
Dabrowska MM, Nazzal K, Wiercinska-Drapalo A. Hepatitis A and hepatitis A virus/HIV coinfection in men who have sex with men, Warsaw, Poland, September 2008 to September 2009.
Euro Surveill. 2011;16(34):pii=19950. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19950

Article published on 25 August 2011

We describe the epidemiology and characteristics of hepatitis A among men who have sex with men (MSM) who had been hospitalised due to the infection in Warsaw, Poland, from September 2008 to September 2009. A total of 50 men were analysed; their median age was 28 years (range: 17–43). None had travelled to hepatitis A-endemic regions during the six months before hospitalisation nor had they been vaccinated against hepatitis A. Of the 50 men, 40 had been tested before hospitalisation or on admission for the presence of anti-HIV antibodies: six were coinfected with HIV. The six HIV-positive MSM were significantly older than those who were HIV negative – median age: 37 years (range: 26–43) versus 28 years (range: 17–43); p=0.02. No difference in disease severity or the duration of hospitalisation was observed, however, between the two groups. Our study underlines the need to screen MSM for hepatitis A and to vaccinate them against hepatitis A. Given the ages of the MSM in our study, we recommend that this be carried out in Poland when the MSM are aged 20–35 years. This should apply not only to MSM with multiple casual partners but also to those in monogamous relationships.

Introduction

Hepatitis A virus (HAV) is one of the most common causes of acute hepatitis worldwide [1,2]. This usually self-limiting disease does not lead to chronic hepatitis [3], but older age, excessive alcohol intake and chronic or simultaneous infection with hepatitis B virus (HBV) may sometimes increase the severity of hepatitis A [4]. Faecal-oral transmission is the most common route; however, transmission by unprotected oral or anal sex among men who have sex with men (MSM) may also occur [5].

In Europe, during the last century, the largest hepatitis A outbreaks occurred during the two world wars [6]. However, in developed countries today, outbreaks are sporadic and have been limited to certain regions or cities, as in the hepatitis A-related food-borne outbreaks in Austria and southern Italy in 2008 [7,8].

Notification of hepatitis A is mandatory in Poland. Physicians from infectious diseases units who confirm the infection by detection of anti-HAV IgM antibodies in the blood of a person with clinical symptoms or family physicians who suspect the infection on the basis of clinical symptoms or by laboratory findings of acute hepatitis are obliged to report the case, using a specific questionnaire, to the National Institute of Public Health, which is responsible for surveillance in Poland. As there is no screening for hepatitis A in the country, only symptomatic patients who are hospitalised are offered an anti-HAV IgM test.

All cases of viral hepatitis had been registered in Poland since 1951, but it is only since 1997 that hepatitis A cases have been reported separately. Before 1997, the incidence of hepatitis A in Poland was high, ranging from 155 per 100,000 population to 196 per 100,000 population [9]. Due to progressive improvements in working and living conditions in the country – particularly the gradual introduction of sanitation and hygiene measures – the incidence of hepatitis A decreased considerably in the years following 1997. The incidence in 1997 was 10.47 per 100,000 population; in 1998, it was 5.20 per 100,000 population and in 2005, 1.18 per 100,000 population [9].

In 2008 and 2009, an unexpected increase in the number of reported hepatitis A cases was seen. A total of 208 cases were detected in 2008 and 652 in 2009, whereas the number in 2007 was 36 (data collected by the National Institute of Public Health). In 2008 and 2009, some of the cases were refugees (n=76) [10] and some had travelled to North Africa (n=14) (unpublished data). However, this does not account for the almost six-fold and more than 18-fold increase in the number of hepatitis A cases reported in 2008 and 2009, respectively, compared with 2007. The total case numbers in 2008 and 2009 led to an incidence of 0.55 per 100,000 population and 1.72 per 100,000 population, respectively, while in 2007 it was 0.09 per 100,000 population and in 2006, 0.29 per 100,000 population.

In 2006 and 2007, around 50% of the reported hepatitis A cases in Poland were linked to recent travel (during the last six months) to hepatitis A-endemic areas, such as in Africa (Egypt, Ghana and Tunisia), South America (Brazil, Peru and Mexico), Asia (India, Nepal and Indonesia) and Europe (Turkey, Russia and Ukraine). They were reported in late summer or autumn: in 2006, 45% of cases were travel related; in 2007, it was 53% [11].

In Poland, anti-hepatitis A vaccination is not obligatory for children or adults (and must be paid for by individuals). The vaccine is generally only proposed to people before travel to a hepatitis A-endemic region.

Presently in Poland, hepatitis A is a disease of adolescents and young adults (aged 20–30 years). In the 1970s, the highest incidence was observed in children aged 7–9 years [9]. However, after 1997, the highest incidence was in adults aged 25–29 years (1.12 per 100,000 population in 2008). The incidence in males (all ages) in 2008 was 0.68 per 100,000 population; in females (all ages), it was 0.42 per 100,000 population. It is of note that the most affected group has become men between 25 and 29 years old. In 2008, the incidence in this group ranged from 0.50 per 100,000 population in men aged 40–44 years to 1.73 per 100,000 population in men aged 25–29 years and this trend seems to have continued in 2009 [10,12,13].

Hepatitis A in MSM has been reported in Europe. For instance, in a hepatitis A outbreak in Denmark in 2004, the incidence rate in Copenhagen was 23 per 100,000 men above 17 years old; the median age of the men was 41 years (range: 19–73). Of the 107 men affected, 68 (64%) were reported to be MSM [14]. Similarly, in Northern Ireland, between October 2008 and July 2009, of the 38 cases in a hepatitis A outbreak, 36 were men, whose median age was 29 years. Of the 36 men, 26 were MSM [15]. Unfortunately, data on the incidence of hepatitis A in MSM in Poland are sparse, as information about sexual orientation is not routinely collected when physicians report cases of hepatitis A through the surveillance system.

The objective of our study was to describe the epidemiology of hepatitis A among MSM in Warsaw, the capital, which has the largest number of MSM in Poland. At the end of April 2011, 14,474 people in the country were registered with HIV infection: 78% (n=11,289) were male. More than 50% (about 7,300) of all HIV-infected persons were MSM [16]. We also present the characteristics (age, travel history and possible route of transmission) of MSM with HAV/HIV coinfection.

Methods

We included men who identified themselves as MSM who had been hospitalised in Warsaw's Hospital for Infectious Diseases due to clinical manifestations of hepatitis A (described below) and elevated serum levels of liver enzymes and bilirubin (tested in the Department of Hepatology and Acquired Immunodeficiences, Warsaw Medical University) during September 2008 to September 2009.

According to the national case definition, a case of hepatitis A was defined either as a person with clinical symptoms of acute hepatitis such as jaundice, fever (temperature above 38°C), abdominal pain, loss of appetite, malaise, nausea and vomiting, and with elevated serum levels of liver enzymes in standard laboratory tests, and with possible faecal-oral transmission of hepatitis A in anamnesis (e.g. eating fast food, travel to hepatitis A-endemic regions or contact with a person with jaundice), or as a person with clinical symptoms, elevated serum levels of liver enzymes and who tested positive for anti-HAV IgM.

The clinical diagnosis of hepatitis A in all studied MSM was confirmed by detection of anti-HAV IgM antibodies in serum, using the Vitros 3600 immunoassay system, at the time of admission to the hospital [17]. All 50 hepatitis A positive MSM were also tested on admission for HBV (by detection of hepatitis B surface antigen (HBsAg) and anti-HBV IgM), hepatitis C virus (HCV, by detection of anti-HCV antibody and HCV RNA), cytomegalovirus (CMV, by detection anti-CMV IgM) and Epstein–Barr virus (EBV, by detection of anti-EBV IgM).

Liver function tests – measuring serum levels of aminotransferases, alanine phosphatase, gamma-glutamyltrasferase activity and total bilirubin using the Vitros 3600 immunoassay system – were performed for every MSM in the study. Each patient was tested several times during their hospitalisation: for all patients, the levels on admission were the highest observed.

Questions about possible exposure to HAV (e.g. travel abroad, food consumption and (non-sexual) contact with a person with symptoms of hepatitis A) and on sexual orientation were asked on hospital admission, as a part of the clinical examination.

The non-parametric Mann–Whitney U test was used to compare the studied groups (HIV-positive and HIV-negative MSM with hepatitis A). A p value of less than 0.05 was considered statistically significant. Statistical analyses were performed using Statistica 8.0 (StatSoft Inc., United States).

For this retrospective observational study, we needed no ethical approval because all laboratory tests carried out were part of the routine management of acute hepatitis.

Results

A total of 50 MSM hospitalised due to hepatitis A were included in the study. None had travelled to known hepatitis A-endemic regions in the world during the six months before hospitalisation. In their anamneses, all mentioned one or more episodes of risky sexual intercourse (e.g. with multiple casual partners and/

or without a condom) during the six months before hospitalisation.

The median age of the 50 MSM was 28 years (range: 17–43). The six HAV/HIV-coinfected patients were significantly older (median age: 37 years; range: 26–43) than the 34 HIV-negative men (median age: 28 years; range: 17–43); p=0.02. However, we saw no difference in the level of liver enzymes and of bilirubin, or in the duration of hospitalisation between both groups (Table).

For 13 of the MSM, their regular sexual partner had been diagnosed as being infected with HAV during the six months before the MSM in the study had been hospitalised. None of the 50 MSM or their regular partners had medical records of having been vaccinated against hepatitis A. No data on hepatitis A status (vaccination or past infection) were available for their casual partners.

All 50 MSM were tested for HBV and hepatitis C virus on admission: none had an acute or chronic infection. However, two of the men had record of an acute hepatitis B infection in the past.

The CD4 cell count of the six HIV-positive MSM, determined when hepatitis A had been diagnosed, was low: the median was 300/µL (range: 106–406/µL).

In all six cases of HAV/HIV coinfection, the men had had several episodes of unprotected oral and/or anal sex with their partners during the six months before hospitalisation.

All patients obtained the same treatment for their symptoms of hepatitis A (e.g. replacement of fluids lost as a result of vomiting or diarrhoea) and all made a full recovery.

Discussion and conclusion

Data from the European Centre for Disease Prevention and Control (ECDC) concerning hepatitis A in Europe in 2008 showed the predominance of male cases in all age groups under 65 years of age (the highest being in age groups 0–4 years and 15–44 years) [18].

As described earlier, hepatitis A in MSM has been reported in Europe [14,15]. In our study, none of the MSM had been vaccinated against hepatitis A. Although our study was small, the level of vaccination was much lower than that described in Diamond et al.'s study of MSM who attended public venues in King County, Washington, United States: of the MSM who reported that they had received at least one dosis of HAV vaccine and had HAV IgG upon serologic testing, only 15% had been vaccinated against HAV [19].

The route of HAV transmission was difficult to determine for the patients in our study because of the lack of blood samples from their sexual partners (to analyse if they were infected with the same HAV strain). However, in 13 of the MSM, we suspect that their infection had occurred as a result of oral and/or anal sex, because their regular sexual partner had been infected with HAV during the previous six months; however, there is no molecular evidence to support this. Unfortunately, none of the MSM had been vaccinated against hepatitis A after their partners had been diagnosed with the infection.

The most important aspect of our study is in providing data about hepatitis A in MSM in Warsaw: such data are scarce in Poland as data on sexual orientation are not routinely collected by the national surveillance system for cases of hepatitis A. We also identified six cases of HAV/HIV coinfection in the studied MSM. Given their low CD4 cell count, it is clear that in all six coinfected men, their HIV infection was not controlled.

Table

Duration of hospitalisation and laboratory results for liver function tests[a] for HIV-positive (n=6) and HIV-negative (n=34) men who have sex with men with hepatitis A, Warsaw, Poland, September 2008–September 2009

Variable	HIV-positive MSM	HIV-negative MSM	P value
	Median (range)	Median (range)	
Number of days hospitalised	12 (5–34)	10 (4–17)	0.43
AST (IU/mL)	929 (222–2,124)	1,730 (157–6,338)	0.18
ALT (IU/mL)	2,368 (600–5,000)	2,943 (717–7,898)	0.29
ALP (IU/mL)	182 (76–241)	215 (74–401)	0.47
GGTP (IU/mL)	418 (125–999)	273 (122–542)	0.40
Total bilirubin (µM/L)	101 (72–168)	109 (28–301)	0.51
Number of MSM	**6**	**34**	**–**

ALT: alanine aminotransferase; ALP: alanine phosphatase; AST: aspartate aminotransferase; GGTP: gamma-glutamyl transferase; IU: international units; MSM: men who have sex with men.

[a] On admission to hospital, peak levels.

Our study had some important limitations, such as the small number of MSM and the inclusion of hospitalised cases only. Moreover, the length of hospitalisation as a measure of disease severity may not be ideal, given the subjectivity involved in determining how long a patient stays in hospital. Unfortunately, we could not compare the results of our study of MSM in Warsaw with those from other regions of the country because data on MSM in Poland are sparse. Thus the results of our study cannot be generalised to the rest of the country. Despite its limitations, however, to the best of our knowledge, this is the first study in Poland to look at hepatitis A and sexual orientation.

In conclusion, given incidence of hepatitis A in MSM in Poland and given that MSM are a particular risk group, we underline the need for hepatitis A screening and anti-HAV vaccination for every MSM. Given the ages of the MSM in our study, we recommend that this be carried out in Poland when the MSM are aged 20–35 years. This is important not only for those with multiple casual partners but also for those in monogamous relationships.

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