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Outbreak of *Shigella sonnei* infection in Norway linked to consumption of fresh basil, October 2011

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We report a *Shigella sonnei* outbreak of 46 cases that occurred in Norway during October 2011. Two municipalities were involved. A large cluster (42 cases) was concentrated in north Norway, while a small cluster (4 cases) occurred in the south-east region. Epidemiological evidence and traceback investigations have linked the outbreak to the consumption of imported fresh basil. The product has been withdrawn from the market. No further cases have been reported since 25 October.

Outbreak description

On 9 October 2011, the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health was informed by the Municipal Medical Officer and the Local Food Safety Authority in Tromsø (northern Norway) about an unusually high number of cases of gastrointestinal disease caused by *Shigella sonnei*.

A delicatessen and catering company located in the centre of Tromsø received several complaints from customers who had fallen ill with gastrointestinal symptoms after having eaten food items from there.

On 14 October, a small cluster of cases who had not been to Tromsø were reported and the outbreak was classified as national.

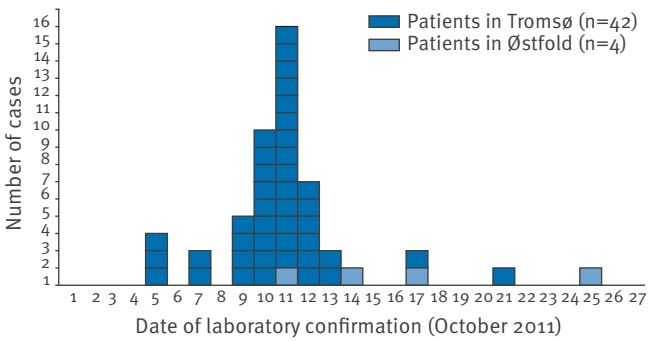
An outbreak case was defined as a person with gastrointestinal symptoms with laboratory confirmed infection with *S. sonnei* with indistinguishable multiple-locus variable number of tandem repeats analysis (MLVA) profiles in Norway after 1 October 2011.

As of 2 November, 46 cases have been reported (Figure). The outbreak-MLVA profile had not previously been seen in Norway. The cases are distributed in two clusters: 42 cases live in Tromsø in the north of Norway or had been to Tromsø within the incubation period of the disease (up to seven days) [1,2], and four cases live in a municipality (Østfold) in south-east Norway, without any connection to Tromsø. None of the cases had travelled outside the country. All cases are adults (aged 19–84 years) and males and females are equally affected. No new cases have been reported since 25 October.

Shigellosis is the third most frequent bacterial gastrointestinal infection reported in Norway. In the last 10 years, between 120 and 190 cases have been registered annually (about 2.5 per 100,000 inhabitants to 4.0 per 100,000 inhabitants). Most of the cases (80–90%) are travel related. *S. sonnei* is the most frequent species identified (64% of all isolates in 2010) [3].

Figure

Laboratory-confirmed outbreak cases due to *Shigella sonnei* infection, Norway, October 2011 (n=46)



Investigation into the outbreak

On 9 October, the Municipal Medical Officer in Tromsø initiated an investigation in collaboration with the local Food Safety Authority in order to identify the source of the outbreak. The local Food Safety Authority interviewed 38 of the 42 laboratory-confirmed cases in Tromsø: most of them had participated in social events after 30 September where food provided by the catering company in question had been served. Of the 38 cases interviewed, 36 reported to have eaten pesto containing basil. The catering company provided a complete list of all the events for which they had served food from 30 September, which was followed up by the local Food Safety Authority.

One of those events was a baptism ceremony banquet celebrated on 1–2 October. Of the 50 attendees, 10 people became sick with gastrointestinal symptoms. Among the sick, three had laboratory-confirmed infection with *S. sonnei*. In collaboration with the local authorities, the Norwegian Institute of Public Health performed a cohort study among the attendees of the banquet in order to identify risk factors for illness. A link to a web-based questionnaire designed to collect information on demographics, symptoms and food exposures from the menu was sent by email to 42 of the attendees. All of them responded. Preliminary results from the cohort study show that two products, a pesto and a soup, were independently associated with illness. However, only the pesto was delivered by the catering company and could be linked to the other cases occurring in Tromsø.

On 11 October, the first case in the small cluster of four cases in a municipality in the south of the country with MLVA profiles indistinguishable identical to the Tromsø profile was identified. Three had been served food containing basil in a specific restaurant.

Traceback investigations of ingredients in the pesto served in Tromsø are still ongoing. The same distributer that provided the fresh basil to the catering company in Tromsø also delivered fresh basil to the restaurant implicated in the second cluster in south-east Norway. The distributor imported this herb from a country outside the European Union and has voluntarily withdrawn it from the market. The National Veterinary Institute analysed samples of pesto and other ingredients from the catering company in Tromsø. Samples available for analysis have been negative. An epidemic intelligence information system (EPIS) enquiry has been posted to determine whether other European countries have observed a similar increase in cases infected with *S. sonnei*. So far, no other countries have reported any recent increase in cases that can be linked to this outbreak.

Conclusion and recommendations

We report an outbreak of *S. sonnei* in Norway, linked to imported basil used fresh in pesto. The ingestion of very few organisms (10–100) is sufficient to cause infection [4]. Pesto usually contains a substantial amount of basil. Thus, if this herb is contaminated with the bacteria, ingesting very small quantities of pesto can lead to a high risk of getting the infection.

Each year there are a considerable number of outbreaks of shigellosis around the world due to consumption of contaminated food. The contamination of foods with *Shigella* usually results from contaminated irrigation water, infected food handlers or improper preparation [5,6]. The sources of many shigellosis outbreaks have been traced to the ingestion of raw or fresh vegetables [6]. In Norway, for example, iceberg lettuce was incriminated in a *S. sonnei* outbreak in 1994 [7]. Previous domestic outbreaks of *S. sonnei* infection in Scandinavia have been also linked to imported products [8].

Food handlers with gastrointestinal symptoms should avoid involvement with the preparation, management and transport of food while they are symptomatic in order to prevent spread of the pathogen, and they should also adhere to appropriate hygiene and handwashing routines. Control measures to protect fresh vegetables from air, soil or water contamination should be ensured.

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Case report: Tick-borne encephalitis in two Dutch travellers returning from Austria, Netherlands, July and August 2011

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Tick-borne encephalitis (TBE) is not endemic in the Netherlands and diagnostics are seldom requested. Here, we report about the rare event of TBE in two Dutch travellers returning from Austria in July and August 2011. This report serves to create awareness among physicians to consider travel-related TBE in their differential diagnosis of patients with neurological disease returning from TBE virus endemic regions and to promote awareness among professionals advising travellers.

In the Netherlands, tick-borne encephalitis virus (TBEV) is not endemic. Diagnostic requests for TBEV are not common, and, between 2006 and 2011, these averaged 17 per year (range 9–25) for the laboratory of the Netherlands Centre for Infectious Disease Control, which is one of the two laboratories in the Netherlands that perform TBEV diagnostics. Diagnosed imported tick-borne encephalitis (TBE) is also very rare in the Netherlands. A positive anamnesis for a stay in a TBEV endemic area (incubation period of typically 7–14 days) and a tick bite are epidemiological parameters that should lead to undertake a confirmative diagnosis based on positive TBEV IgM and IgG responses. Based on these criteria, in the five-year period from 2006 to 2010, only one person was diagnosed as an acute TBEV case based on IgM and IgG seroconversion (data not shown). The following report describes two cases of tick-borne encephalitis in travellers returning to the Netherlands in the summer of 2011.

Case reports

Case 1. In mid-July 2011 a Dutch woman in her late fifties presented to the general practitioner emergency post. After a few days of dizziness, non-productive cough and fear of noise, she had, on the day of admission, an acute fever (39 °C) accompanied with severe headache and muscle pain. Muscle weakness, nausea and vertigo were also present. The weeks before she had made an extensive camping trip in Austria (Prems, Pongau, Imst), followed by two days in the Black Forest (Germany), where she detected a tick on her right buttock. The patient indicated that the tick probably had been present for days. The tick was removed 17 days before admission to the hospital. On clinical examination no neurological abnormalities were detected. On the right buttock there was a red induration of 1 cm in diameter on the location of the tick bite. During the ten days of admission, headache and muscle pain were the main complaints. Serology for borreliosis was negative. Potential infection with TBEV was suspected in an early phase based on the clinical picture (fever and headache) in combination with the registration of a tick-bite and the recent leisure activities in Austria, where TBE is endemic. Serology, using an enzyme-linked immunosorbent assay (Immunozyme FSME IgM and IgG, Progen, Heidelberg, Germany) on a serum sample taken four days upon onset of disease showed high titres of TBEV specific IgM and IgG antibodies, indicative of an active TBEV infection. The patient recovered gradually. One month later she only had some insignificant headache and hypersensitivity to noise.

Case 2. At the end of August, a Dutch man in his early forties was admitted to the hospital with a severe headache, fever and fatigue. He also complained about impaired hearing and blurred vision. He had returned from holidays in Austria (Carinthia) 16 days prior to admission. In Austria, the patient had been bitten by a tick in the neck. About a week later the man got fever and severe headaches. He received antibiotic treatment (amoxicillin/clavulanic acid) in Austria and showed recovery. Upon his return in the Netherlands a few days later his headache and fever returned and became more severe. Based on travel history, fever and headache, meningoencephalitis was suspected as differential diagnosis. Cerebrospinal fluid (CSF) and serum

were sampled. CSF analysis showed 62 leucocytes per µL (norm: < 5 leucocytes per µL) with a dominance of monocytes (56 per µL (norm: 15–45%). CSF total protein was 909 mg/L (norm: 150–500 mg/L). Herpes simplex virus, varicella zoster virus, enterovirus and parechovirus PCR on CSF were negative. Serology for Lyme disease was negative as well. Initial treatment consisted of ceftriaxone and aciclovir. Infection with TBEV was suspected based on the leisure activities in Austria and the nature and severity of the symptoms. Antibody testing on CSF was positive for TBEV IgG but not for IgM. His serum showed high titres of TBEV specific IgM and IgG antibodies, indicative of a recent TBEV infection. Subsequently, aciclovir and cefriaxone were stopped. The patient recovered slowly. However, seven weeks after his admission to the hospital he still suffers of headache, severe fatigue, hearing impairment, blurred vision and memory problems.

Background

TBEV is the causative agent of TBE, the most important viral tick-borne disease in Europe [1]. The virus is transmitted to humans through bites of infected ticks within minutes of the tick bite. Transmission through consumption of raw milk from infected dairy cattle has also been described. Human-to-human transmission does not occur [1,2-4]. The occurrence of most human cases coincides with the occurrence of questing ticks, roughly from May to November.

The TBEV species comprises three distinct genetic lineages; the European (TBEV-Eu), Far-Eastern (TBEV-FE) and Siberian (TBEV-Sib) subtypes [5]. TBEV-Eu has been isolated in Europe, TBEV-Sib in the Urals, Siberia and far-eastern Russia, and TBEV-FE in far-eastern Russia, Japan and China. Co-circulation of different subtypes has been recorded for Finland, Latvia, Lithuania and Estonia [1,2-4]. The severity of disease with TBEV aetiology varies depending on the causative subtype.

Germany and Austria are endemic countries for TBEV with low incidence (less than one case per 100,000 inhabitants). However the incidence and virus circulation in Germany varies considerably between regions, with non-endemic areas in the north, but in the south defined TBE risk areas in Baden-Württemberg, Bavaria, Hesse, Thuringia and Rhineland-Palatinate [6]. In Austria, the incidence is low due to the high vaccination coverage (88%) but the circulation of TBEV is high [7].

Discussion

The two confirmed tick-borne encephalitis cases reported here were related to travel to Austria, although infection in the Black Forest in Baden-Württemberg Germany cannot be excluded for case 1. They coincide with an increased circulation of TBEV elsewhere in Europe in 2011 probably as a consequence of favourable weather conditions for both increased tick densities and human exposure [8].

The number of leisure trips from the Netherlands to Austria in the summer season has been stable for the period 2006–2010 and accumulates to an average of 470,000 trips per year. Yearly, 5.8 million overnight stays are spend by Dutchmen in Austria in trips lasting more than eight days in the summer season and 0.6 million overnight stays while travelling through [9]. It has been estimated that an unvaccinated tourist spending four weeks in a highly endemic region in Austria has a risk of contracting TBE of one per 10,000 man-months of exposure [10].

Applying this figure to the total number of overnight stays of Dutchmen in Austria during the summer season, one could conclude that clinical TBE cases are likely to be underdiagnosed in the Netherlands, namely three diagnosed cases of acute TBE in the period 2006–2011 versus a roughly estimated yearly incidence of 20 cases. However such estimations are complicated by the fact that TBE incidence is the result of complex interactions between several risk factors including the level of circulation in provinces visited (which vary considerably in endemic countries) and the outdoor recreational behaviour.

An estimated 2,800 TBE cases were prevented in Austria in the period 2000–2006 through vaccination but Austria remains a high-risk area for unvaccinated tourists, and an increase in disease incidence in unvaccinated individuals has been observed [11,12].

The case descriptions in this report highlight the importance of considering TBE as a travel disease which should be taken into account when travelling to endemic areas in Europe in general. An inventory by the European Network for Imported Viral Diseases (ENIVD), for the period between 2004 and 2009, identified the Baltic states, Slovenia and the Czech Republic, as countries with high risk areas (yearly incidence of more than five cases per 100,000 inhabitants). Russia, Switzerland, Sweden and Slovakia showed yearly incidences over one while the incidences in Austria, Germany, Hungary, Poland, Norway and Finland were rather low (under one per 100,000). Recent years show an overall geographic expansion in all directions of TBEV in Europe [7]. Interestingly, in September 2011 Sweden reported record numbers of TBE patients for 2011 [8].

In the period 2006–2009 the number of trips from the Netherlands to European countries with high risk areas accumulated to 9.3 million and a mere 10.6 million trips when countries with a lower public health impact of TBEV are considered [9]. This underlines the necessity of an increased awareness for TBEV-related risks among physicians, professionals advising travellers and travellers. Outdoor activities in forest areas with dense undergrowth are related to an increased risk for TBEV infection [1,2-4]. TBEV is partly preventable by wearing trousers, long sleeves and tick-repellents [10]. Early removal of ticks does not prevent disease [1]. The

most effective measure is vaccination. The Scientific Working Group of Tick-borne encephalitis stresses the importance of raising awareness in non-endemic regions for travel-related TBE and recommends tick-borne encephalitis virus vaccination for Europeans travelling to areas of TBEV risk [12,13]. Whether vaccination of travellers is cost-effective remains to be seen, but as a minimum, travellers to TBEV endemic areas should be educated about the health risks and the possible preventive measures.

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Seroprevalence of herpes simplex virus type 1 and type 2 in Thuringia, Germany, 1999 to 2006

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The prevalence of herpes simplex virus (HSV) type-specific IgG was determined in sera taken in 1999 to 2006 from 1,100 children aged 0–18 years, 800 blood donors and 200 pregnant women in Thuringia, Germany, using tests based on the HSV glycoproteins (g) gG. By the age of 10–12 years, HSV-1 IgG prevalence reached 57.3%, rising to 69.3% by the age of 16–18 years and to 78.0% by the age of 28–30 years. Between 2.7% and 4.7% of the children aged up to 15 years had HSV-2 antibodies, increasing to 7.3% at the age of 16–18 years and to 13.6% among adults. The prevalence of HSV-1 antibodies among girls was significantly lower than among boys and a significantly higher prevalence of HSV-2 IgG in women than in men was detected. The reduced incidence of HSV-1 infections during childhood, especially in girls, has to be followed up since a higher number of primary HSV-2 infections may result. Between 2.7% and 4.7% of all children tested seemed to acquire HSV-2 by intrauterine or neonatal infection. We also compared the use of gG-1 with gC-1: the agreement of 97.2% between the two ELISAs suggests that gG-1 and gC-1 can be considered equivalent antigenic targets.

Introduction

Herpes simplex virus (HSV) is one of the most common pathogens affecting humans: it can remain latent lifelong in sensory ganglia and can be reactivated periodically. Infections may result in substantial physical and psychological morbidity. There are two types, HSV-1 and HSV-2, which differ not only genetically but also in the principal route of transmission, the body site predominantly affected, seroprevalence and rate of reactivation [1]. Both types are mainly transmitted by direct contact. In HSV-1 infection, the body above the waist is predominantly affected; in HSV-2, it is mainly below the waist. For HSV-1, primary infections occur mostly during infancy and childhood, after maternal antibodies have disappeared in the first year of life. In contrast, HSV-2 mainly affects adolescents and adults. Excretion of the virus from herpetic lesions (mainly lips and genitals) in symptomatic people with recurrent infections and excretion of the virus in the saliva and genitals of asymptomatic people are regarded as the most important source of the virus [2].

Unlike HSV-1, HSV-2 is predominantly acquired through sexual activity [3] and causes the great majority of genital herpes [4]. In persons with past primary HSV-1 infection, the risk of acquiring HSV-2 is probably reduced since most antibodies cross-react between HSV-1 and HSV-2 [5]. Although no clinical signs can be recognised in many people with HSV-2 infections, as is also the case for HSV-1 infections, asymptomatic people can shed the virus, and thus there is a substantial risk of viral transmission to their sexual partners [6]. Even though the risk of vertical viral transmission is lower than that for acquiring primary infection, recurrent genital herpes must be regarded as the most common cause of neonatal infections [7]: up to 85% of neonatal herpes infections are caused by HSV-2 [8], which are associated with a poor prognosis [9].

HSV type-specific seroprevalence has been extensively studied in many countries all over the world. Seroprevalence rates of HSV-1 of between 50% and 85% or more have been shown in adults from developed countries, such as Germany, Spain and Norway, in the last two decades [3,10]. For HSV-2, seroprevalence varied as a function of age, sex, number of lifetime sexual partners and socio-economic status [3,10]. In Germany, the prevalence of HSV-1 IgG antibodies reached levels of more than 90% in adults during 1996 to 1998, whereas about 15% of adults possessed IgG antibodies to HSV-2 [10]. Further studies revealed that the prevalence of antibodies to both HSV types has not changed over the last two to three decades in Germany [11]. However, only a few studies have analysed the time of HSV seroconversion during infancy, childhood and adolescence. In particular, little is known about the

seroprevalence of HSV-2 in children: seroprevalence in childhood is often used to estimate the incidence of intrauterine and neonatal HSV-2 infections. Recent studies have shown that nearly one third of children in Sweden [12] have been infected with HSV-1 and there is a gradual development of antibodies throughout childhood [3,12]. In contrast, HSV-2 antibodies have been reported to appear generally after sexual debut [3,13].

The envelope glycoproteins (g) G of HSV-1 (gG-1) and HSV-2 (gG-2) are known to be type-specific antigens [14] and have been used in nearly all conclusive HSV-1 and HSV-2 seroprevalence studies [3]. Recently, gC-1 was shown to be an equivalent antigenic target for type-specific diagnosis of HSV-1 infections [15].

As there are few data on the prevalence of HSV antibodies in Germany, we carried out a seroepidemiological study to determine the HSV-1 and HSV-2 antibody prevalence in children, adults and pregnant women in the German federal state of Thuringia. For detection of HSV-1-specific antibodies, results from two enzyme-linked immunosorbent assays (ELISAs) based on the detection of gG-1 or gC-1 were compared for 2,100 serum samples collected during 1999 to 2006.

Methods

Patients and serum samples

In this study, 1,100 sera from infants, children and adolescents aged 0 to 18 years, (hereafter referred to as children aged 0 to 18 years), 800 sera from blood donors aged 19 to 30 years and 200 pregnant women aged between 17 and 40 years were included. These samples had been taken for routine serological tests, for example, medical check-ups or determination of immune status, during 1999 to 2006. For all people whose samples were included, the clinical or laboratory data did not indicate the presence of infectious diseases.

For the blood donors and pregnant women, informed oral consent for use of their sera was obtained. For children aged up to 18 years, consent was not sought. According to the Central Ethical Committee of Germany [16], when residual samples are used for research studies, patient consent is not required in exceptional cases and the effort needed to obtain the consent is not justifiable. In addition, the Ethical Committee of the University of Jena, Germany, approved the study.

Samples from those aged 18 years or under lived in Erfurt, the capital of Thuringia, and its rural surroundings. A total of 844 (76.7%) of the 1,100 whose samples were selected were healthy and 256 (23.3%) had adiposity or growth disorders. The anonymised samples were selected by block randomisation from remainders of sera that had been submitted to the laboratory during 1999 to 2006 (770 sera from 1999 to 2000 and 330 sera between 2001 and 2006).

Anonymised sera from the blood donors were selected by block randomisation during 1999 and 2000 from healthy donors living in the region of Erfurt.

Serum samples from the pregnant women were taken from those who delivered at their local district hospital (in Thuringia) between January 1999 and January 2000. The women were enrolled consecutively in the study. All were healthy and routine laboratory parameters were normal.

Testing of sera

Sera were stored in aliquots at –20 °C without interruption until tested for antibodies to HSV-1 and HSV-2. All sera were brought to room temperature immediately before the test was carried out.

Antibody testing was carried out blindly in groups of 90 serum samples. Sera were tested in parallel using HerpeSelect 1 ELISA IgG (Focus Diagnostics, United States), which uses gG-1, and Anti-HSV-1-gC1-ELISA IgG (Euroimmun, Germany) for determination of HSV-1 IgG, as well as HerpeSelect 2 ELISA IgG (Focus Diagnostics, United States) for determination of HSV-2 IgG.

All samples with discordant results in the gG-1 and gC-1 ELISAs were retested twice and the most frequent result (including the original test result) was accepted.

All samples with equivocal results in the gG-1 and gC-1 ELISAs were retested twice. All samples that still gave equivocal results in the gG-1 ELISA after retesting were assessed using immunoblot *recom*Line HSV-1 & HSV-2 IgG (Mikrogen, Germany).

All sera with equivocal results in the HSV-2 ELISA were retested twice. Those that still gave equivocal results after retesting were analysed using immunoblot *recom*-Line HSV-1 & HSV-2 IgG, as were all sera that were positive, to avoid false-positive results, as results from other studies suggests that HSV-2 ELISAs may results in false-positive results [17].

ELISAs

The HerpeSelect 1 ELISA IgG and HerpeSelect 2 ELISA, which have been licensed by the United States Food and Drug Administration, use recombinant gG-1 and gG-2 antigens, respectively. The sensitivity and specificity of these ELISAs for samples from sexually active adults have been shown to be 91.2% (HSV-1) to 96.1% (HSV-2) and 92.3% (HSV-1) to 97% (HSV-2) [18,19]. All tests were carried out manually. Samples were considered positive if the index value was greater than 1.1. Negative samples had an index value of less than 0.9 and those with index values between 0.9 and 1.1 were considered equivocal. Seroprevalence was calculated only on the basis of the ELISAs using gG-1 and gG-2 and the retest results using an immunoblot assay, where appropriate.

The Anti-HSV-1-gC1-ELISA IgG uses affinity chromatography-purified gC1 isolated from HSV-1 [15]. Testing of sera was carried out manually. A standard curve based on the extinction values of three calibration sera containing 2, 20 or 200 relative units (RU) per mL was used to the calculate results. Samples with a cut-off value of 22 RU/mL or higher were considered positive, 16–22 RU/mL equivocal and below 16 RU/mL negative. On the basis of the results, we analysed the agreement between the gG-1 and gC-1 ELISAs.

Immunoblot assay

The immunoblot assay *recom*Line HSV-1 & HSV-2 IgG is based on nitrocellulose membranes blotted with purified recombinant gG-1 and gG-2 as well as with proteins common to both types of HSV. The test was performed manually. According to the manufacturers [20], the HSV-1 assay has been shown to have 99% (in routine diagnostic samples) to 100% (in samples from blood donors) sensitivity and 88.7% (routine diagnostics) to 94.6% (blood donors) specificity. For the HSV-2 immunoblot, the sensitivity was given as 75.0% (blood donors) to 93.8% (routine diagnostics) and the specificity as 92.5% (routine diagnostics) to 100% (blood donors).

Statistical analysis

A sample size of about 150 subjects per pre-specified age group was planned to assure that a two-sided 95% confidence interval (CI) for the prevalence of HSV antibodies would extend at most 8% from the observed value for a prevalence range of 5–95%.

Antibody prevalence was calculated using the number of seropositive cases divided by the number of all subjects tested. Assuming a binominal distribution, the two-sided exact 95% CI was calculated.

Differences in antibody prevalence between the sexes, age groups as well as between pregnant women and female blood donors were evaluated by logistic regression odds ratios based on Wald statistics. The level of significance was 0.05 (two-sided). We used SAS V9.2 software for statistical analyses.

The amount of agreement between gG-1 and gC-1 ELISAs was computed using the number of sera with concordant results divided by the number of sera tested.

Results

Prevalence of HSV-1 IgG

Data on the prevalence of IgG antibodies against HSV-1 in the samples tested are shown in Table 1. In the children aged 0–18 years tested, the overall prevalence of antibodies against HSV-1 was 47.6% (95% CI: 44.6–50.6). During the first year of life, the prevalence was 48.0% (95% CI: 40.9–55.2). It then fell to 19.3% (95% CI: 13.3–26.6) among those aged 2–3 years. The prevalence increased to 39.3% (95% CI: 31.5–47.6) among the

Table 1

Prevalence of IgG antibodies against HSV-1 in children (aged 0–18 years), adults (blood donors aged 19–30 years) and pregnant women (aged 17–40 years), Thuringia, Germany, 1999–2006 (n=2,100)

Age group in years	Male		Female		Male and female	
	Number of positive samples/ total number	Percentage (95% CI)	Number of positive samples/ total number	Percentage (95% CI)	Number of positive samples/ total number	Percentage (95% CI)
Infants, children and adolescents						
0–1	63/116	54.3 (44.8–63.6)	33/84	39.3 (28.8–50.5)	96/200	48.0 (40.9–55.2)
2–3	23/90	25.6 (16.9–35.8)	6/60	10.0 (3.8–20.5)	29/150	19.3 (13.3–26.6)
4–6	26/78	33.3 (23.1–44.9)	33/72	45.8 (34.0–58.0)	59/150	39.3 (31.5–47.6)
7–9	43/84	51.2 (40.0–62.3)	24/66	36.4 (24.9–49.1)	67/150	44.7 (36.6–53.0)
10–12	40/64	62.5 (49.5–74.3)	46/86	53.5 (42.4–64.3)	86/150	57.3 (49.0–65.4)
13–15	46/84	54.8 (43.5–65.7)	37/66	56.1 (43.3–68.3)	83/150	55.3 (47.0–63.4)
16–18	42/61	68.9 (55.7–80.1)	62/89	69.7 (59.0–79.0)	104/150	69.3 (61.3–76.6)
Total	**283/577**	**49.0 (44.9–53.2)**	**241/523**	**46.1 (41.7–50.5)**	**524/1,100**	**47.6 (44.6–50.6)**
Adults (blood donors)						
19–21	45/67	67.2 (54.6–78.2)	90/133	67.7 (59.0–75.5)	135/200	67.5 (60.5–73.9)
22–24	66/90	73.3 (63.0–82.1)	92/110	83.6 (75.4–90.0)	158/200	79.0 (72.7–84.4)
25–27	70/100	70.0 (60.0–78.8)	74/100	74.0 (64.3–82.3)	144/200	72.0 (65.2–78.1)
28–30	76/97	78.4 (68.8–86.1)	80/103	77.7 (68.4–85.3)	156/200	78.0 (71.6–83.5)
Total	**257/354**	**72.6 (67.6–77.2)**	**336/446**	**75.3 (71.1–79.3)**	**593/800**	**74.1 (70.9–77.1)**
Pregnant women						
17–40 **Total**	–	–	**164/200**	**82.0 (76.0–87.1)**	–	–

HSV: herpes simplex virus.

4–6 year-olds, to 44.7% (95% CI: 36.6–53.0) among the 7–9 year-olds and to 57.3% (95% CI: 49.0–65.4) among the 10–12 year-olds. In adolescents aged 13–15 years, 55.3% (95% CI: 47.0–63.4) had been infected; in the16–18 year-olds, the prevalence was 69.3% (95% CI: 61.3–76.6).

The Figure shows the age- and sex-specific prevalence of HSV-1 IgG in the children and adults (blood donors) tested.

Among the blood donors tested, the overall prevalence of HSV-1 IgG antibodies was 74.1% (95% CI: 70.9–77.1). Among those aged 19–21 years, it was 67.5% (95% CI: 60.5–73.9) and increased to values between 72% (95%: 65.2–78.1) and 79.0% (95% CI: 72.7–84.4) among the 22–30 year-olds.

In the pregnant women tested, the prevalence of HSV-1 IgG was 82.0% (95% CI: 76.0–87.1).

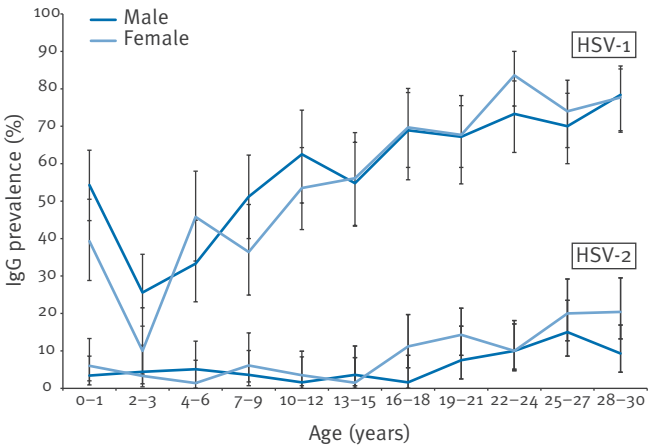
Statistical analysis demonstrated significantly lower prevalence of HSV-1 antibodies in children up to the age of 15 years in comparison with that of the blood donors ($p<0.001$). Adjusted for age, the prevalence of HSV-1 antibodies among girls aged between 0 and 18 years was significantly lower than among boys of the same age ($p=0.039$).

Prevalence of HSV-2 IgG

The prevalence of antibodies against HSV-2 in the samples tested is shown in Table 2. In the children tested, the overall prevalence was 4.2% (95% CI: 3.1–5.5). In the first year of life, the prevalence was 4.5% (95% CI: 2.1–8.4). In children aged 2–3 years up to those aged 13–15 years, the lowest value of 2.7% (95% CI: 0.7–6.7) was seen in those aged 10–15 years and the highest, 4.7% (95% CI: 1.9–9.4), in those aged 7–9 years. The prevalence increased to 7.3% (95% CI: 3.7–12.7) among the 16–18 year-olds and to values between 10.0% (95% CI: 6.2–15.0) and 17.5% (95% CI: 12.5–23.5) among the 19–30 year-old blood donors, with the lowest value in those aged 22–24 years and the highest in those aged 25–27 years. The age- and sex-specific prevalence of HSV-2 IgG in the children and adults (blood donors) tested is shown in the Figure.

Figure

Age- and sex-specific prevalence of IgG antibodies against HSV-1 and HSV-2 in children (aged 0–18 years) and adults (blood donors aged 19–30 years), Thuringia, Germany, 1999–2006, (n=1,900)



HSV: herpes simplex virus.

The bars show the 95% confidence intervals for the point estimates.

The overall prevalence of HSV-2 IgG was 13.6% (95% CI: 11.3–16.2) in the blood donors and 18.0% (95% CI: 12.9–24.0) in the pregnant women.

Of 191 people who were found to be HSV-2 positive 147 (77.0%) were coinfected with HSV-1. Only 8/46 (5/26 female and 3/20 male) children 0–18 years, 26/109 (15/71 female and 11/38 male) blood donors and 10/36 pregnant women were seropositive for HSV-2 alone (data not shown).

Statistical analysis revealed a significantly lower prevalence of HSV-2 IgG in the children (0–18 years) compared with adults (blood donors) ($p<0.001$). Adjusted for age, a significantly higher prevalence of HSV-2 IgG in women than in men was detected ($p=0.021$).

Agreement between the HSV-1 gG-1 and gC-1 ELISAs

Table 3 shows the results of retesting sera that gave discordant or equivocal results using the two HSV-1 ELISAs.

Of the 2,100 sera tested in this study, 71 showed equivocal results in either of the HSV-1 ELISAs after having been repeated twice and were excluded from the comparison of both ELISAs. There was an overall agreement of 97.2% (1,973/2,029) between the results of the gG-1 and gC-1 ELISAs.

When age groups were compared, the agreement between both tests was 95.6% (177/185) in infants aged 0–1 year, 96.7% (421/435) in children aged 2–9 years, 96.8% (424/438) in those aged 10–18 years, 97.9% (759/775) in the blood donors and 98.0% (192/196) in the pregnant women.

Of the 40 sera that gave equivocal results in the HSV-1 gG-1 ELISA after having been repeated twice, 37 were negative by immunoblot assay.

Retesting of sera in the HSV-2 ELISA

Of the 32 sera that gave equivocal results in the HSV-2 gG ELISA, after having been repeated twice, 28 were negative by immunoblot assay (Table 3).

After retesting of sera that were positive in the HSV-2 gG-2 ELISA (n=201), 191 (95%) were also positive in the HSV-2 immunoblot.

Discussion

Seroprevalence studies of HSV-1 and HSV-2 are vital for a better understanding of the public health importance of disease due to HSV. In this study, we were particularly interested in determining the HSV seroprevalence of children up to the age of 18 years, since there is little known about this. However, a limitation of the study is that the collection of sera over a seven-year period may mask changes in seroepidemiology.

As expected, the prevalence of antibodies against HSV-1 and HSV-2 was significantly lower in children and adolescents than in adults; however, for HSV-1, this concerned children only up to the age of 15 years. After the disappearance of maternal antibodies in the first year of life, there was an increase in HSV-1 IgG prevalence to 20% by the age of 2–3 years, to 40% by 4–6 years, to almost 60% by 10–12 years, to 70% by 16–18 years and to about 80% by the age of 28–30 years. These data add to previous findings from the

Table 2

Prevalence of IgG antibodies against HSV-2 in children (aged 0–18 years), adults (blood donors aged 19–30 years) and pregnant women (aged 17–40 years), Thuringia, Germany, 1999–2006 (n=2,100)

Age group in years	Male		Female		Male and female	
	Number of positive samples/ total number	Percentage (95% CI)	Number of positive samples/ total number	Percentage (95% CI)	Number of positive samples/ total number	Percentage (95% CI)
Infants, children and adolescents						
0–1	4/116	3.4 (0.9–8.6)	5/84	6.0 (2.0–13.3)	9/200	4.5 (2.1–8.4)
2–3	4/90	4.4(1.2–11.0)	2/60	3.3 (0.4–11.5)	6/150	4.0 (1.5–8.5)
4–6	4/78	5.1(1.4–12.6)	1/72	1.4 (0.0–7.5)	5/150	3.3 (1.1–7.6)
7–9	3/84	3.6 (0.7–10.1)	4/66	6.1 (1.7–14.8)	7/150	4.7 (1.9–9.4)
10–12	1/64	1.6 (0.0–8.4)	3/86	3.5 (0.7–9.9)	4/150	2.7 (0.7–6.7)
13–15	3/84	3.6 (0.7–10.1)	1/66	1.5 (0.0–8.2)	4/150	2.7 (0.7–6.7)
16–18	1/61	1.6 (0.0–8.8)	10/89	11.2 (5.5–19.7)	11/150	7.3 (3.7–12.7)
Total	**20/577**	**3.5 (2.1–5.3)**	**26/523**	**5.0 (3.3–7.2)**	**46/1,100**	**4.2 (3.1–5.5)**
Adults (blood donors)						
19–21	5/67	7.5 (2.5–16.6)	19/133	14.3 (8.8–21.4)	24/200	12.0 (7.8–17.3)
22–24	9/90	10.0 (4.7–18.1)	11/110	10.0 (5.1–17.2)	20/200	10.0 (6.2–15.0)
25–27	15/100	15.0 (8.6–23.5)	20/100	20.0 (12.7–29.2)	35/200	17.5 (12.5–23.5)
28–30	9/97	9.3 (4.3–16.9)	21/103	20.4 (13.1–29.5)	30/200	15.0 (10.4–20.7)
Total	**38/354**	**10.7 (7.7–14.4)**	**71/446**	**15.9 (12.6–19.7)**	**109/800**	**13.6 (11.3–16.2)**
Pregnant women						
17–40 **Total**	–	–	**36/200**	**18.0 (12.9–24.0)**	–	–

HSV: herpes simplex virus.

Table 3

Retesting of sera with discordant or equivocal results in HSV-1 and HSV-2 ELISAs, Thuringia, Germany, 1999–2006

Type of result	Number of sera[a]	
	HSV-1 (gG-1 and gC-1)	HSV-2 (gG-2)
Discordant		
Discordant results between the gG-1 and gC-1 ELISAs (initial test)	133/2,100	–
Discordant results between the gG-1 and gC-1 ELISAs after repeating the ELISAs twice	124/133	–
Equivocal		
Equivocal results in the intial ELISA	**HSV-1 gG-1 only** 40/2,100	32/2,100
Equivocal results in the ELISA after repeating the ELISA twice	40/40	32/32
Results after retesting by immunoblot assay	3/40 positive 37/40 negative	4/32 positive 28/32 negative

ELISA: enzyme-linked immunosorbent assay; g: glycoprotein; HSV: herpes simplex virus.

[a] Number of sera with the relevant results. A total of 2,100 were initially tested.

German population, using a relatively small number of sera from infants, children and adolescents [10].

A considerable delay of HSV-1 infection during childhood has been reported from Finland, where only 17% of children at the age of eight years had antibodies to HSV-1 [21]. Surprisingly, the prevalence of HSV-1 antibodies among girls was lower than among boys in our study. Most studies have not shown significant differences between girls and boys. Our findings are in contrast to the study from Davidovici et al., which demonstrated significantly higher HSV-1 seroprevalence in females aged 10–19 years in Israel [22]. In keeping with the findings from Israel, our study suggests that HSV-1 infection is being acquired later in life. This is in contrast to data from the data from the United States, where 68% of the population aged over 12 years had HSV-1 antibodies [23], The overall age-specific HSV seroprevalence rates in childhood obtained from Brazil are 1.5 times higher [24] and the figures from Tanzania are two to three times higher [25] than in this study, from one part of Germany. This variation might reflect regional sex-specific differences in exposure to the virus. Since previous infection with HSV-1 may protect against HSV-2 infection or attenuate the severity of disease, lower antibody prevalence among girls may result in a higher number of primary HSV-2 infections that are mostly localised in the genital tract in sexually active individuals. Hence, a reduced incidence of HSV-1 infections during childhood and adolescence, especially in girls, has to be followed up in further HSV-1 seroprevalence studies.

A low prevalence of HSV-2 antibodies of between 2.7% and 4.7% was seen in children aged up to 15 years. In those aged 16–18 years, the prevalence increased to 7.3%, indicating a rising incidence of primary HSV-2 infections, probably due to the start of sexual activity. Our data suggest that between 2.7% and 4.7% of all children seem to acquire HSV-2 antibodies in response to intrauterine or neonatal infection. Such infections may be clinically unapparent or not recognised clinically [26]. It should not be forgotten, however, that the low HSV-2 IgG prevalence might also be caused, at least partially, by false-positive test results because of the limited specificity of HSV-2 antibody tests [17]. A review of the literature suggests that the incidence of neonatal diseases caused by both types of HSV ranges widely, from 5 to 31 per 100,000 live births [27]. Relatively high prevalence rates of HSV-2 infection in Tanzanian children suggest that non-sexual transmission of HSV-2 (e.g. person-to-person contact by fingers and hands contaminated with the virus) might also be a reason for HSV-2 seropositivity in childhood [25].

In our study, adults (blood donors) aged between 19 and 30 years had an HSV-2 seroprevalence of 13.6%. There was a significantly higher prevalence of HSV-2-specific antibodies among women (16%) than among men (11%) and in pregnant women, the prevalence was 18%. However, the difference in prevalence between pregnant women and female blood donors was statistically not significant. The seroprevalence data of HSV-2 in blood donors in this study are comparable with findings from other parts of Germany [10]. The considerably lower seroprevalence rate of 8.9% among pregnant women from Stuttgart (Germany) [28] can only be interpreted in the context of the different serological methods used and differences between the populations tested. These factors have also to be considered for the interpretation of data from several countries, where HSV-2 seroprevalence ranged from 4% (in England and Wales) to 24% (in Bulgaria) [29].

Our finding of a higher seroprevalence of HSV-2 among women than among men is in agreement with several other studies [10,30,31]. This may result from the differential role of sex on clinical presentation since men appear to have a higher tendency of asymptomatic HSV-2 infection [30]. In contrast, infected women are more often symptomatic, which may stop them from having sexual intercourse. This can lead to higher rates of male to female viral transmission.

There is a strong consensus in the literature that tests for HSV type-specific IgG, such as immunoassays or immunoblots that are based on gG-1 and gG-2, are the most accurate for discriminating between infections with HSV-1 or HSV-2 in seroprevalence studies and in serological diagnostics [32-34]. A recent study revealed that gC-1 and gG-1 may be comparable antigenic targets for the serodiagnosis of HSV-1 infections [15]. To our knowledge, the study presented here is the first comprehensive serosurvey that includes both gG-1- and gC-1-based ELISAs. The high level of agreement between the gG-1 and the gC-1 ELISAs, in all age groups, demonstrates a high degree of concordance between reactivities against gG-1 and gC-1. Therefore, the gC-1-specific ELISA appears to be an equivalent alternative to gG-1 tests for the determination of type-specific IgG antibodies against HSV-1.

Conflicts of interest

S. Schmitt is employed by SanofiPasteur MSD, T. Scheper and S. Saschenbrecker are employed by the Institute of Experimental Immunology (affiliated to Euroimmun AG), and M. Motz and E. Soutschek by Mikrogen.

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Influenza surveillance during the post-pandemic influenza 2010/11 season in Greece, 04 October 2010 to 22 May 2011

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In this manuscript, we summarise the experience of Greece during the post-pandemic influenza season 2010/11 from 04 October 2010 to 22 May 2011. The spread of the disease and its impact were monitored using multiple surveillance systems, such as sentinel surveillance, virological surveillance and all-cause mortality surveillance. We also focus on the characteristics of laboratory-confirmed severe influenza cases who required admission to an intensive care unit (ICU) (n=368), and/or with a fatal outcome (n=180). The influenza-like illness rate reported from sentinel surveillance started rising in early January 2011 and peaked between 31 January and 6 February 2011. The total number of ICU admissions was higher in the post-pandemic influenza season than during the pandemic period causing a lot of pressure on ICUs. The overall population mortality rate due to influenza A(H1N1)2009 was higher than during the pandemic period (15.9 vs 13.2 fatal cases per million, p=0.087). Our data suggest that the severity of clinical illness in the first post-pandemic influenza season was comparable or even higher than during the pandemic.

Introduction

Between May 2009 and May 2010, Greece experienced two waves of influenza A(H1N1)2009 transmission, a small one in July–August 2009, where cases occurred mostly in areas of the country, which were related to popular tourist destinations, and a larger one that took place all over the country in late autumn, peaking at the end of November 2009. During the pandemic period, there were 18,230 laboratory-confirmed cases, 294 admissions to intensive care units (ICUs) and 149 deaths associated with pandemic influenza virus, a figure that corresponds to one of the highest mortality rates reported in Europe [1-6].

Given the potential for worsening in the clinical severity of influenza during the post-pandemic influenza season, as was the case for previous influenza pandemics [7-9], it was critical to continue surveillance with a focus on severe cases and their clinical characteristics. The United Kingdom (UK) was the first country in Europe to report increased influenza activity as of week 49, 2010, along with an unusual increase in ICU admissions and in the use of extracorporeal membrane oxygenation (ECMO) facilities [10-14].

During the pandemic period, an enhanced ad-hoc surveillance system for severe influenza cases had been in place in Greece [3,4]. Following events in the UK, the same system was reactivated in December 2010. It involved direct reporting to the Hellenic Centre for Disease Control and Prevention (HCDCP) of all laboratory-confirmed influenza cases who were admitted to an ICU, and those with a fatal outcome. In addition, investigators made daily follow-up phone calls to the treating physicians of all patients with laboratory-confirmed influenza who were hospitalised in an ICU.

In Greece, influenza is annually monitored through the routine sentinel surveillance system, which became operational in 1999. The sentinel surveillance system, which covers approximately three percent of the total Greek population in the 2010/11 influenza season, provides data representative of the national population, and consists of three separate networks: (i) private physicians, (ii) primary healthcare centres' physicians, and (iii) physicians of the Social Security Institute (IKA). These networks are coordinated by the HCDCP. During the post-pandemic influenza season (2010/11), a total of 240 physicians (general practitioners, internists, or paediatricians) participated in the sentinel surveillance system. Physicians contributing to this system

reported, on a weekly basis, the number of patients with influenza-like illness (ILI) examined, as well as the total number of consultations. The later number served as a denominator to derive the proportion of ILI patients among the total number of consultations. Based on historical data from the sentinel surveillance system, the influenza season in Greece typically starts in January and lasts through March or April, with a peak in late winter (usually February).

In addition, all-cause mortality was monitored as part of the European Mortality Monitoring (EuroMoMo) project. The objective of this project is to develop a Europe-wide mortality monitoring system for detecting excess deaths related to possible public health threats across Europe, such as influenza [15].

In Greece, influenza vaccination is offered free of charge each year to people in high-risk groups. For the 2010/11 influenza season, the national immunisation campaign started in October 2010 and recommended the trivalent influenza vaccine to persons with chronic conditions, elderly people aged over 60 years, health-care workers and caregivers. Unfortunately, a low rate of vaccine uptake was achieved. A telephone survey estimated that, when the influenza epidemic peaked in the community in early February 2011, the vaccination coverage among the general population stood at just 5.9 percent [16].

This report summarises data from influenza surveillance in Greece during the post-pandemic 2010/11 influenza season. We focus on the characteristics of laboratory-confirmed cases who required admission to an ICU, and of those with a fatal outcome, reported until 22 May 2011.

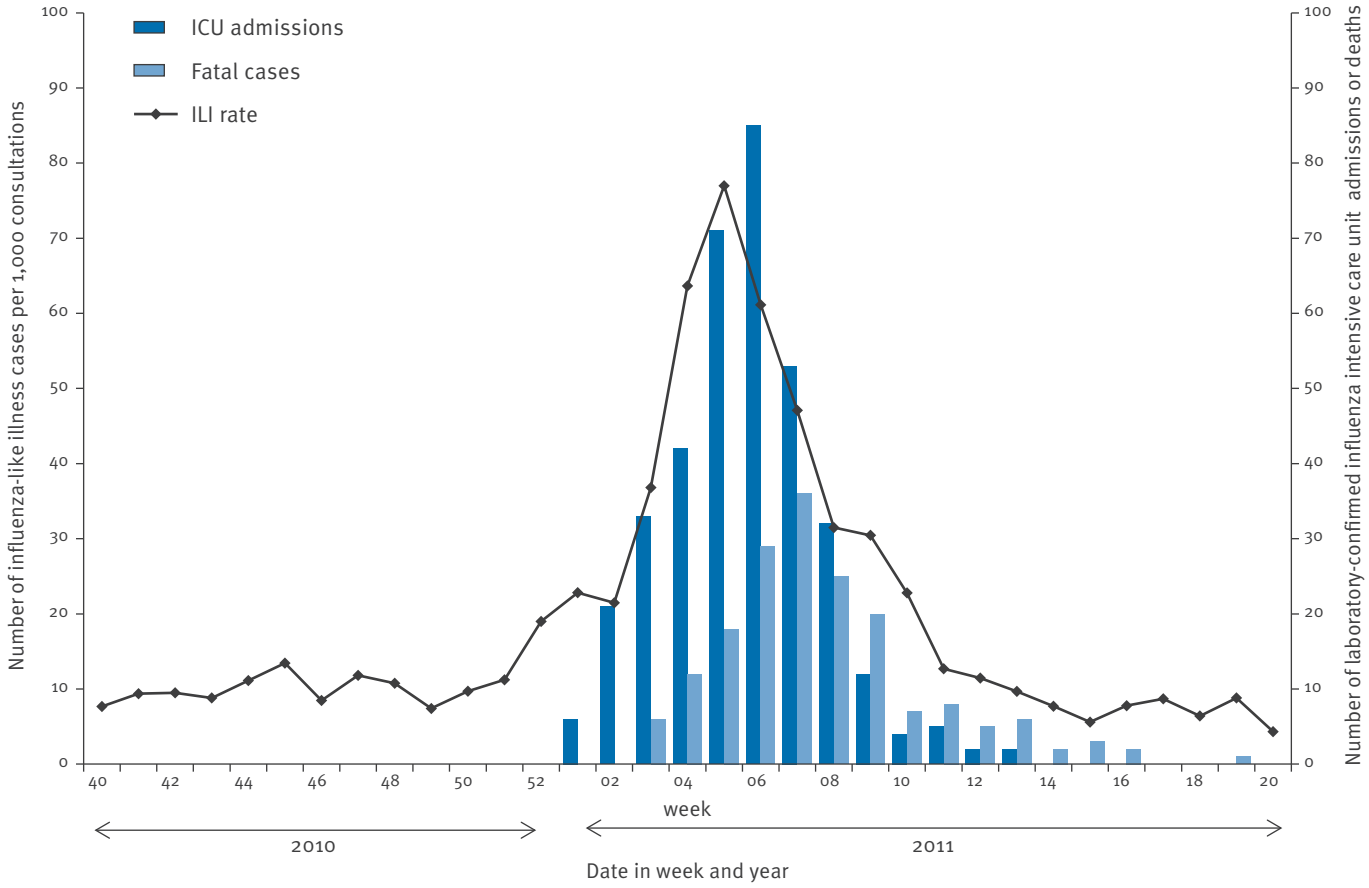
Methods

Patients, whose data were recorded, were all laboratory-confirmed influenza cases (as determined by real-time RT-PCR) who were admitted to an ICU, as well as those with a fatal outcome.

The data collected for each case, using a standardised form, were: demographic characteristics (age, sex), dates of admission to the hospital and the ICU, the time course of illness including the date of symptom onset, underlying conditions, complications, use of mechanical ventilation support (dates of intubation and extubation), and antiviral treatment.

Figure 1

Epidemic curves based on surveillance data from sentinel influenza-like illness consultation rates, intensive care unit admissions (n=368), and fatal cases (n=180), Greece, 04 October 2010–22 May 2011



According to the HCDCP's guidelines, physicians needed to maintain a high level of clinical suspicion for influenza and had to consider influenza in the differential diagnosis of cases admitted to an ICU with severe respiratory disease, such as respiratory failure, chronic obstructive pulmonary disease (COPD) exacerbation, or acute respiratory distress syndrome (ARDS). In such cases, laboratory testing of nasopharyngeal swab specimens was strongly recommended.

Fatal cases were defined as those associated with influenza infection, provided that the infection was laboratory-confirmed either before or after death. A definitive causal relationship between influenza infection and death was not routinely sought and/or established.

Age-specific mortality rates were calculated using the estimated age-specific population of Greece for 2010 [17].

As part of virological surveillance, nasopharyngeal swab specimens were sent for testing by sentinel and hospital physicians to one of the two national reference laboratories.

The statistical analysis was carried out using the GNU R software [18].

Results

Epidemic curve and surveillance results

Figure 1 presents the surveillance results and epidemic curve during the 2010/11 influenza season, based on surveillance data on (i) sentinel ILI, (ii) ICU admissions, and (iii) fatal cases. The sentinel ILI rate followed a typical seasonal pattern with a peak in the fifth week of 2011. The number of ICU admissions and the number of fatal cases followed similar patterns, peaking in the sixth and seventh week of 2011, respectively.

The results of the comparison of the age distribution of the sentinel ILI cases, between the pandemic and the post-pandemic season, were suggestive of a shift to older ages (median age 13 years, range: 0 to 98 years vs 17 years, range: 0 to 96 years, p<0.001; Figure 2a).

Virological surveillance

The results of virological surveillance are summarised in Table 1. In the period between week 40 2010 and week 20 2011, 13,279 specimens were tested. Of all strains detected, from both sentinel and non-sentinel specimens, 98.2% were influenza A and 1.8% influenza B viruses. Of all influenza A strains subtyped, 25 (0.5%) were A(H3N2) while 5,281 (99.5%) were A(H1N1)2009, indicating that the pandemic influenza A(H1N1)2009 was by far the predominant strain.

Surveillance of intensive care unit admissions and fatal cases

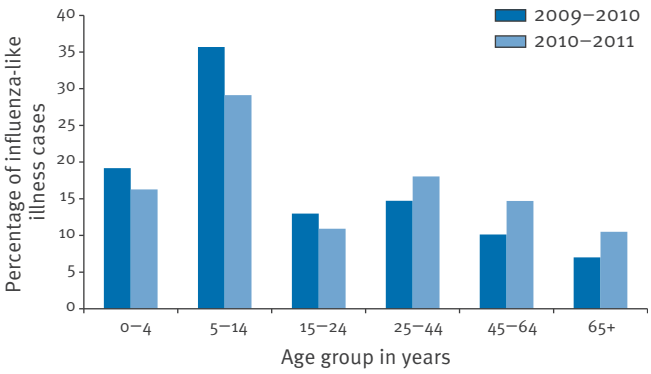
Between 8 January 2011 (when the first case was reported) and 22 May 2011, a total of 368 laboratory-confirmed influenza A(H1N1)2009 cases admitted to an ICU were reported to the HCDCP. Of these cases, 220 survived, 144 died, and four were still hospitalised, at the time when data were extracted for analysis (22 May 2011). The ICU-related case fatality rate was 39%. Another 36 deaths occurred in patients hospitalised in regular wards. All 180 fatal cases were included in the current analysis. On a general population basis, this corresponds to 15.9 (95% CI: 13.7 to 18.4) fatal cases per million.

All laboratory-confirmed cases with a fatal outcome (n=180), were positive for influenza A(H1N1)2009. At the peak of the outbreak, 155 patients were hospitalised at the same time in an ICU.

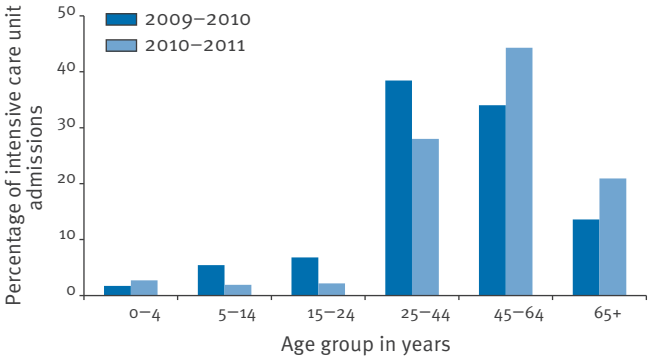
Figure 2

Age distribution of sentinel influenza-like illness cases (n=19,120), intensive care admissions (n=662), and fatal cases (n=329), influenza seasons 2009/10 and 2010/11, Greece

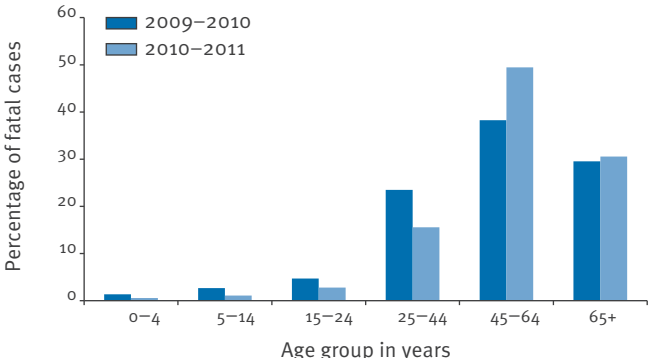
A. Sentinel influenza-like illness cases



B. Intensive care unit admissions



C. Fatal cases



Demographic characteristics

Data on age and sex were available for all cases. The median age of the 368 cases admitted to an ICU was 52 years (range: 3 months to 86 years). Twenty patients (5%) were children aged 18 years or under, and 77 patients (21%) were aged 65 years or older.

The median age of the 180 fatal cases was 56.5 years (range: 2.5 to 96 years). Four of these (2%) were children aged 18 years or under. The majority of cases with a fatal outcome (80%) had been treated in an ICU. Fatal cases treated in an ICU tended to be younger than those treated in the regular wards (median age 56 years, range: 2.5 to 86 years vs 64 years, range: 39 to 96 years, p<0.001).

The age group with the highest mortality rate were adults aged between 45 and 64 years, with a population mortality rate of 30.3 persons (95% CI: 24.3 to 37.3) per million people of the respective age group, followed by the age group of over 65 years with 25.7 persons (95% CI: 19.3 to 33.4) per million.

The results of the comparison of the age distribution of the ICU admitted cases, as well as the fatal cases, between the pandemic period and the post-pandemic influenza season, were suggestive of a shift toward older age groups (p<0.001 and p=0.063, respectively; Figures 2b & 2c).

Of the total 368 cases admitted to an ICU, 207 (56%) were male and 161 (44%) were female. Among cases with a fatal outcome, 106 (59%) were male and 74 (41%) were female. Being male was significantly associated with either admission to an ICU (p=0.010), or death (p=0.012).

Course of disease and antiviral treatment

The time course of illness and antiviral treatment are described in Table 2. Seventeen cases developed influenza-like symptoms while already hospitalised in the ICU for another illness/condition. Three of the 36 fatal cases developed influenza-like symptoms while hospitalised in regular wards. Overall, 357 of the 366 cases with available information (98%) received antiviral treatment. Of 270 for which data was available, 68 (25%) received treatment within 48 hours of symptom onset. The median time from symptom onset to treatment was four days, both for cases admitted to an ICU and for cases with a fatal outcome.

Of the 368 patients admitted to an ICU, 306 (83%) required mechanical ventilation support for a median of 12 days (range: 0 to 76).

Underlying conditions and complications

Patients admitted to an ICU had a median of one underlying condition (118 had no underlying condition, 158 had one, 72 had two, 17 had three, two had four and

Table 1

Results of virological surveillance for influenza, Greece, 04 October 2010–22 May 2011

	Number of specimens (%)
Specimens positive for influenza	5,403 (40.7%)[a]
Specimens positive for influenza type A	5,306 (98.2%)[b]
Specimens positive for A(H1N1) - non pandemic	0 (0.0%)[c]
Specimens positive for A(H3N2)	25 (0.5%)[c]
Specimens positive for A(H1N1)2009	5,281 (99.5%)[c]
Specimens positive for influenza type B	97 (1.8%)[b]
Specimens negative for influenza	7,876(59.3%)[a]
Total number of specimens tested	**13,279 (100.0%)[a]**

[a] Percentage is calculated using total number of specimens tested as denominator.
[b] Percentage is calculated using number of specimens positive for influenza as denominator.
[c] Percentage is calculated using number of specimens positive for influenza type A as denominator.

Table 2

Time course of care-measures for patients with influenza, Greece, 04 October 2010–22 May 2011

	Intensive care unit admissions	Fatal cases
	Median number of days (IQR)	
Symptom onset to hospital admission	**3** (1–5)	**2** (0–5)
Hospitalisation to intensive care unit admission	**1** (0–2)	**1** (0–4)
Duration of stay in intensive care unit	**13** (6–24)	**12** (6–24)
Duration of mechanical ventilation	**12** (6–21)	**11** (5–21)
Symptom onset to start of antiviral treatment	**4** (2–7)	**4** (2–7)

one had five). Underlying disease was equally distributed between the sexes; higher rates were observed in older age groups such as the group aged 60 years and older (p<0.001) (Figure 3).

Fatal cases had also a median of one underlying condition. Two of four children with a fatal outcome had no documented underlying condition, while the majority of cases over the age of 60 years (69 of 74, 93%) had at least one underlying condition. The number of underlying conditions in influenza patients admitted to ICUs or influenza fatal cases, stratified by age group, is shown in Figure 3.

Metabolic disease (including diabetes) and chronic respiratory disease were the most commonly reported long-term underlying conditions among patients admitted to an ICU, while immunosuppresion and chronic cardiovascular disease were the most commonly reported comorbidities among fatal cases (Table 3).

In children (≤18 years), the most commonly reported comorbidity was neurological disease (nine of 20 for children admitted to an ICU and two of four for children with a fatal outcome).

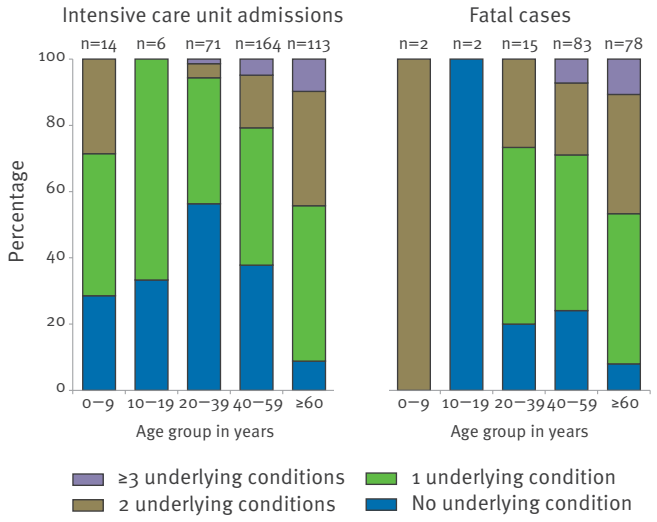
Nine pregnant women required admission to an ICU for influenza. They all had no other risk factors. No death occurred in this group.

Morbid obesity was present in 30 of 358 cases (8%) admitted to an ICU and 18 cases of 173 (10%) with a fatal outcome.

The most commonly reported severe complication of influenza was pneumonia (265 cases, >90% viral) followed by the development of ARDS (250 cases).

Figure 3

Number of underlying conditions in influenza patients admitted to intensive care units (n=368) or influenza fatal cases (n=180), stratified by age group, Greece, 04 October 2010–22 May 2011



Discussion

The burden of severe disease in the first post-pandemic influenza season in Greece was higher than originally expected. The sentinel ILI epidemic curve for this season started around the time that regular influenza season begins in Greece, which is two months later in the winter than the pandemic autumn wave. The epidemic curve for this season was similar in shape and approximately three quarters the size of that observed during the pandemic period [3]. However, when compared with previous influenza seasons (from 2004 onwards) its peak was significantly higher [19]. Regarding the time distribution of ICU admissions, it followed a curve with a peak one week later than the peak of the sentinel surveillance system's curve. This finding is consistent with the fact that increased demand for hospital care usually lags behind the ILI consultation rates by one to two weeks. In our series, the average time from symptom onset to ICU admission was four days.

Table 3

Distribution of underlying medical conditions in influenza patients admitted to intensive care units (n=368) or influenza fatal cases (n=180), Greece, 04 October 2010–22 May 2011

Underlying condition[a]	Proportion of intensive care unit admissions n/N[b] (%)	Proportion of fatal cases n/N[b] (%)
Chronic respiratory disease	75/348 (22%)	39/168 (23%)
Chronic cardiovascular disease	70/353 (20%)	51/171 (30%)
Chronic renal / liver disease	12/342 (4%)	10/163 (6%)
Metabolic disease	86/353 (24%)	47/171 (27%)
Immunosuppression	56/342 (16%)	54/169 (32%)
Neurological / neuromuscular disease	28/346 (8%)	19/168 (11%)
Morbid obesity (body mass index ≥ 40)	30/358 (8%)	18/173 (10%)
Pregnancy	9/368 (2%)	0/180 (0%)

[a] Cases could have more than one underlying medical condition.

[b] Total number of cases or patients for which data were available.

In contrast to ILI, a much higher peak in the distribution of both ICU admissions and fatal cases was observed this season compared to the pandemic period 2009–2010. Nevertheless, data from the EuroMoMo surveillance system operating in Greece did not show any excess all-cause mortality in 2011 up to the end of May [19], as during the pandemic period.

Similar to what happened in the UK in December 2010, during the first weeks of the 2010/11 influenza season, a paradoxically higher number of admissions to ICUs was reported, while the ILI rate was low. Stress on higher-level healthcare facilities when community consultation rates are low is unusual, but this was consistent with the characteristics of the pandemic influenza A(H1N1)2009 strain that causes mild disease in the majority of cases but very severe disease in a very few [20].

The total number of ICU admissions was higher during the post-pandemic influenza season than during the pandemic period and there was more pressure on ICUs. The maximum number of patients hospitalised at the same time in an ICU (155 in the post-pandemic influenza season 2010/11 vs 70 during the pandemic period) corresponds to an approximate 24% coverage of the total ICU bed capacity in the country. However, it is difficult to compare with previous influenza seasons, due to the lack of surveillance for severe influenza cases during these seasons. There was no significant difference in the ICU related case-fatality rates between the two seasons (43% for the pandemic 2009–2010 period vs 39% for the post-pandemic 2010/11 season).

The median age of cases admitted to an ICU was significantly higher than during the pandemic period. This shift to older ages may offer at least a partial explanation for the higher number of fatal cases during this influenza season, since older age has been found to be independently associated with a worse outcome [21].

The most common comorbidities were metabolic and chronic respiratory disease for cases admitted to an ICU, while cardiovascular disease and immunosuppression were most frequently reported among fatal cases. These findings are consistent with published reports from other countries [22,23].

Another noteworthy finding is the presence of neurodevelopmental disease in the paediatric ICU patients. This is in line with data from the pandemic period in Greece, when a large proportion of children and teenagers with a fatal outcome suffered neurological disorders [3]. The findings of a United States (US) study also identify pre-existing neurodevelopmental disorders, as one of the most noticeable risk factors among children who died from pandemic influenza [24].

The delay in initiation of antiviral treatment may be attributed to the fact that patients generally do not seek medical care immediately (median time from onset of symptoms to hospital admission was three days). Data regarding the time course of antiviral treatment are comparable with those reported in a number from other studies [25-27].

Furthermore, the prevalence of morbid obesity both in ICU admitted cases (8%) and in fatal cases (10%) is much higher than the estimate for the adult population of Greece (<1%) [28]. This finding is in agreement with data from other studies [25,29-31], which identify morbid obesity as an independent risk factor for severity of disease associated with the A(H1N1)2009 strain.

The population cumulative mortality in the elderly (≥65 years) was lower than in adults aged 45 to 64 years. Though not statistically significant, this could be the result of lower susceptibility of the oldest group to infection according to serology data for pre-existing immunity from other countries [32].

In conclusion, this report summarises the experience of Greece, during the first post-pandemic influenza season. In contrast to what was observed in countries of the southern hemisphere during this season [33], our data suggest that the severity of clinical illness –as measured by the number of patients admitted to the ICUs, the overall population mortality rate, and the impact on the healthcare system– was comparable or even higher in the first post-pandemic than in the pandemic influenza season. Though there are inherent difficulties in comparing the experiences in diverse countries with varying surveillance systems, sharing data is strongly recommended in order to achieve an improved understanding and capture the range of potential outcomes due to laboratory-confirmed influenza [34]. However, the objectives of severe-end influenza surveillance need to be harmonised across European countries in order to obtain comparable data and facilitate a better design of timely interventions in case of more virulent strains.

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*Erratum:

At the moment of publication, the name of A Andreopoulou was left out of the list of authors. This mistake was corrected on 04 November 2011. We apologise to the authors.

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