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Ongoing measles outbreak, Geneva, Switzerland, January to March 2011

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An outbreak of measles is ongoing in Geneva, Switzerland, since January 2011, in the context of a measles epidemic in neighbouring Rhône-Alpes, France. A total of 41 confirmed cases have been reported, the majority among young adults, many unaware of their non-immune status. There is no large clustering of cases and 14 cases were imported or linked to imported cases. Catch-up vaccination, especially among young adults, may be necessary to prevent further extension of this outbreak.

Background

Between 1 January and 2 March 2011, 58 suspected cases of measles were notified to the Geneva health authorities in Switzerland. Of the 58 suspected cases, 41 (8.9 cases per 100,000 population) were confirmed (by laboratory confirmation or evidence of an epidemiological link) and were living in Geneva. In the previous 18 months (July 2009 to December 2010), only nine confirmed cases had been notified. The last national outbreak of measles in Switzerland lasted from November 2006 to July 2010, with 4,410 reported cases, including 84 in Geneva [1].

A large outbreak of measles is currently being reported in the neighbouring region of Rhône-Alpes, France, where more than 900 cases have been notified from January to mid-February 2011 [2]. Geneva canton (population 464,000) is located on the eastern part of Switzerland and shares 103 km of its border with France and 4.5 km with the rest of Switzerland.

In Switzerland, measles notification has been mandatory since 1999. Physicians should report to local health authorities any patient presenting with maculopapular rash associated with fever and any of the following: cough, coryza or conjunctivitis. Notification of confirmed cases is also mandatory for laboratories. Childhood measles vaccination has been recommended since 1975: the current vaccination policy is to vaccinate twice with measles-mumps-rubella (MMR) at the age of 12 months and 15–24 months (the second dose was introduced in 1996) [3]. The most recent estimate of national coverage among children aged two years is 89.8% for one dose and 74.6% for two doses (unpublished data).

Case definition

A case was defined as a Geneva canton resident presenting with the above clinical symptoms between 1 January and 2 March 2011, with either a detectable titre of measles virus-specific IgM antibodies, detection of measles virus RNA by PCR or a clinically significant increase in measles virus-specific IgG (laboratory-confirmed cases) or evidence of an epidemiological link with a laboratory confirmed case (epidemiologically linked cases).

Case description

Of the 41 cases included in the analysis, 33 were laboratory confirmed (by IgM or PCR) and eight were epidemiologically linked cases. The epidemic curve is presented in Figure 1. An additional 14 notified cases were not included, even though they were diagnosed in Geneva and reported to the health authorities, because they were French residents (n=11) or living in the canton of Vaud (n=3). All worked, attended school or consulted a physician in Geneva. Three suspected cases were reported and later ruled out due to negative serology results.

The male to female sex ratio of the 41 cases was 1:2 and their median age was 20 years (range: 11 months–58 years). Of 35 cases with known immunisation status, 25 had not been vaccinated with MMR vaccine, eight had received one dose and two at least two doses. Cases are presented by age group and vaccination status in Figure 2.

Six patients presented with at least one complication such as pneumonia (n=4), bronchitis (n=1), respiratory failure (n=1) and otitis (n=1). Eight patients were hospitalised: one was in intensive care with respiratory failure, one had pneumonia, three had a general alteration of heath status (in one case, this was associated with hypoxemia and in one, with fever) and two were kept in

for overnight observation. The reason for hospitalisation is still unknown for one patient. No deaths were reported. All but one of the patients with complications or who were hospitalised were adults (median age: 36 years).

Of the 41 cases, 14 were imported (n=8) or epidemiologically linked with an imported case (n=6). These cases came from or were epidemiologically linked to cases from France (n=9), the canton of Vaud (n=4) and, possibly, Argentina (n=1).

Four clusters were identified: in two clusters, there were four cases per cluster, and in two clusters, there were two cases per cluster, giving a total of 12 cases. Transmission occurred in settings such as families (three occurrences), schools (n=2), social contacts (n=4) and the health service (n=1).

Figure 1

Measles cases by importation status and clustering, Geneva, Switzerland, 1 January–2 March 2011 (n=41)

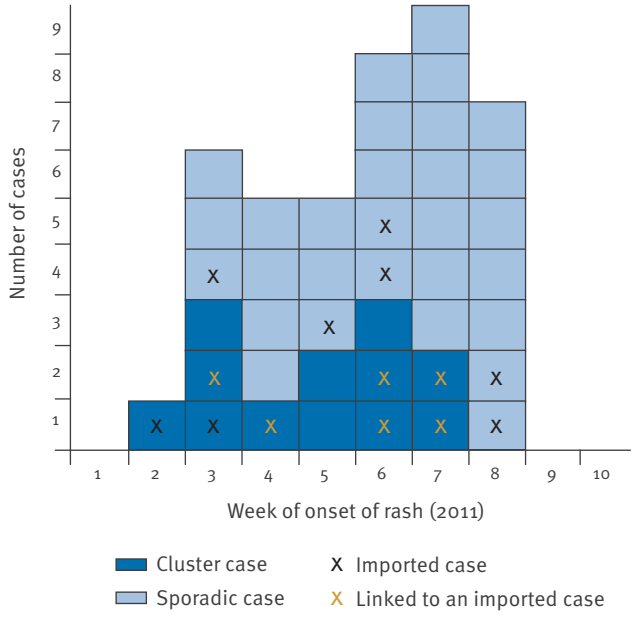
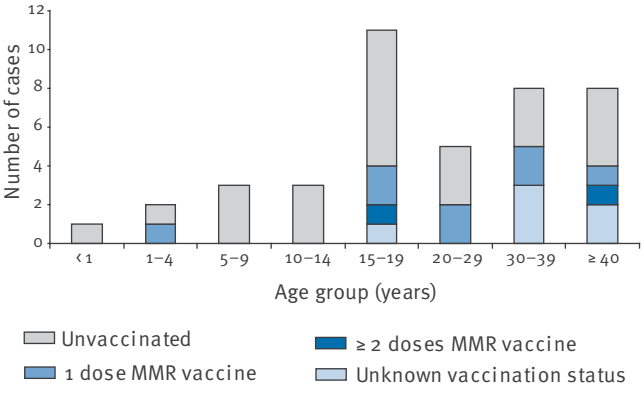


Figure 2

Measles cases by age and immunisation status, Geneva, Switzerland, 1 January–2 March 2011 (n=41)



MMR: measles-mumps-rubella.

Control measures

In Switzerland, notification is mandatory within 24 hours of diagnosis and control measures are implemented as early as possible by local health authorities and school health services without waiting for laboratory confirmation. Cases are isolated at home for four days after the beginning of their rash. Extensive and rapid contact tracing is conducted as an emergency measure so that contacts and relatives of cases can be informed and their vaccination or immunisation status assessed. Control measures are customised and depend on the age, immunisation status of the contact and when the contact took place. They include post-exposure vaccination of unvaccinated or non-immune contacts with recent exposure (less than 72 hours), administration of immunoglobulins to unvaccinated or non-immune pregnant women and newborns less than six months old. Unvaccinated or non-immune siblings, close and classmate contacts are quarantined at home for 18 days, with vaccination at the end of the quarantine period. A total of 20 exposed non-immune persons were quarantined. Five developed measles but there were no subsequent cases; seven are still quarantined.

In addition, Geneva health authorities regularly sent epidemiological updates and practical information by email to local physicians. These include reducing the age of first measles vaccination from 12 to 9 months of age. A press release, individual emails to all Geneva university students, and information letters to directors of schools, day-care centres and crèches have been sent out by the university or school health services. The main message has been targeted to young adults, recommending them to check their immunisation status and be vaccinated if necessary.

Discussion

This outbreak occurred in the context of a large measles epidemic in neighbouring Rhône-Alpes region in France. In addition, cases have also been recently diagnosed in the Lausanne and Basel regions, but very few are being reported in other parts of Switzerland.

The epidemiology of infectious diseases in the canton of Geneva is closely related to its neighbouring regions for obvious economic and geographic reasons. As a consequence, about one third of the cases in the outbreak were imported or related to imported cases, mostly from the bordering department of Haute-Savoie. The 14 cases who were not included in this analysis as they did not reside in Geneva were investigated in collaboration with the local French health authorities.

Most cases in this outbreak were adults, many of whom were not aware of their immune status. There was a delay in the diagnosis of several cases as early presentation of measles can be quite similar to influenza (seasonal influenza peaked in Geneva between weeks 1 and 7 of 2011). In some instances, there were multiple consultations before measles was diagnosed. However, only one healthcare-related case has been documented so far.

Control measures have been implemented early for all cases including those whose measles diagnosis has not yet been confirmed. Post-exposure vaccination is often ineffective because it is implemented too late. As secondary attack rate in unvaccinated household members is high [4], quarantine of non-immunised relatives and close contacts and classmates is enforced and has proven effective in previous outbreaks in Geneva [5]. None of the five quarantined contacts who developed measles transmitted the virus to others.

Although national MMR vaccination coverage remains below the threshold required for measles elimination in Switzerland [6,7], it is somewhat higher in Geneva. In 2007, 89.7% of 28-month-old infants had received two doses of measles vaccine [7-9] and in 2009, the corresponding figure was 91.7%, according to the most recent analysis of the Geneva vaccination database [10], in June 2010. Progressive accumulation of non- or insufficiently immunised persons is therefore inevitable and explains this outbreak, along with multiple introduction of infectious patients into the Geneva community.

Conclusion and recommendations

Fairly high MMR vaccination coverage in children as well as early and effective prevention measures have probably contributed to reducing the magnitude of this outbreak, as indicated by the absence of any large clusters of cases so far.
However, the influx of cases from neighbouring regions may continue and the potential for extension of the outbreak is substantial.

Catch-up vaccination is critical to prevent further extension of this outbreak, especially among young adults, particularly students. Careful scrutiny of the situation should continue as well as close collaboration with the neighbouring health authorities.

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Increasing West Nile virus antibody titres in central European plasma donors from 2006 to 2010

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We analysed by neutralisation assay 55 intravenous immunoglobulin preparations produced from human plasma collected in three central European countries, specifically Austria, Germany and the Czech Republic, from 2006 to 2010. The preparations from 2009 and 2010 contained increasing titres of neutralising antibodies against West Nile virus (WNV) in the absence of reported human WNV cases in these countries.

Introduction

Clinical cases of West Nile virus (WNV) infection in the European Union (EU) have so far been limited to sporadic outbreaks (Table), while serological studies in sentinel horses and birds, as well as humans, have shown WNV circulation for decades, particularly in the southern EU Member States [1]. However, as exemplified by the WNV outbreak in Greece in summer 2010, the pathogenicity of this virus for humans can change. A study in 2007 detected 1% (4 of 392) WNV seropositivity in human serum samples collected from asymptomatic individuals living in Greece [2], whereas in 2010, WNV caused the second largest outbreak of human infections recorded in the EU since the outbreak in Romania in 1996 [3].

In order to assess how many people in central European countries may have been exposed to the virus, including individuals with subclinical disease, we analysed the WNV neutralising antibody titre of intravenous immunoglobulins (IVIG), a blood product containing pooled immunoglobulins from the plasma of more than a thousand donors from Austria, Germany and the Czech Republic. The suitability of this approach for assessing the cumulative number of WNV infections in a large donor population has recently been demonstrated through the use of IVIG to follow the spread of WNV in the United States [35].

Methods and results

Fully validated neutralisation assays were used to determine WNV and tick-borne encephalitis virus (TBEV) neutralisation titres (NT_{50}) in 55 individual lots of IVIG manufactured from plasma pools (≥1,000 donors) collected in three EU countries (KIOVIG, Baxter), Austria (ca. 40-45%), Germany (ca. 40-45%) and to a lesser extent the Czech Republic (ca. 15%). The IVIG lots tested were produced in the years 2006 (n=10), 2007 (n=9), 2009 (n=20) and 2010 (n=16). For the year 2008, IVIG lots produced exclusively from EU plasma were not available for testing. The NT_{50} were determined, at least in duplicate, as described [35], using WNV strain 385-99, lineage 1 or TBEV strain Neudoerfl. When tested against WNV, IVIG samples were used undiluted and titrated on Vero cells (ECACC 84113001); against TBEV, samples were initially diluted 1:5 and titrated on A549 (ATCC CCL-185) cells. After seven days, each well was assessed for virus-induced cytopathic effect, and the NT_{50}, i.e. the reciprocal dilution resulting in 50% virus neutralisation, is reported as mean±standard deviation of two or more replicates (Figure 1B). Plotting the WNV neutralisation titres of the respective IVIG lots against the year of product release, a statistically significant increase over the years 2006 to 2010 was observed ($p=0.0269$ one way ANOVA and $p=0.004$, post test for linear trend; Figure 1A).

To specifically discriminate antibodies induced by WNV infections of EU plasma donors from possibly cross-reactive antibodies against TBEV, a Flavivirus related to WNV and widely endemic in central Europe, the TBEV neutralisation capacity of the IVIG lots was also determined, after the first lots with higher WNV activity had been detected. This was done for 30 of the 55 IVIG lots (Figure 1B). We assessed a potential contribution of TBEV neutralising antibody titres to the measured WNV antibody titres by correlation analysis and found a significant correlation ($r^2=0.5$, $p=0.002$) between the TBEV and WNV neutralisation titres for 19 of 30 IVIG lots analysed (Figure 1B, open circles). The apparent WNV neutralisation capacity of the majority of IVIG lots produced from plasma collected in Austria, Germany and the Czech Republic therefore resulted, at least partially, from cross reactive antibodies as induced by the wide-spread use of TBEV vaccines or TBEV infections. The IVIG lots produced from plasma collected more recently, six lots in 2009 and five lots released for 2010 until April, did not fit this correlation. In general, plasma collection pre-dates release of IVIG lots

Table

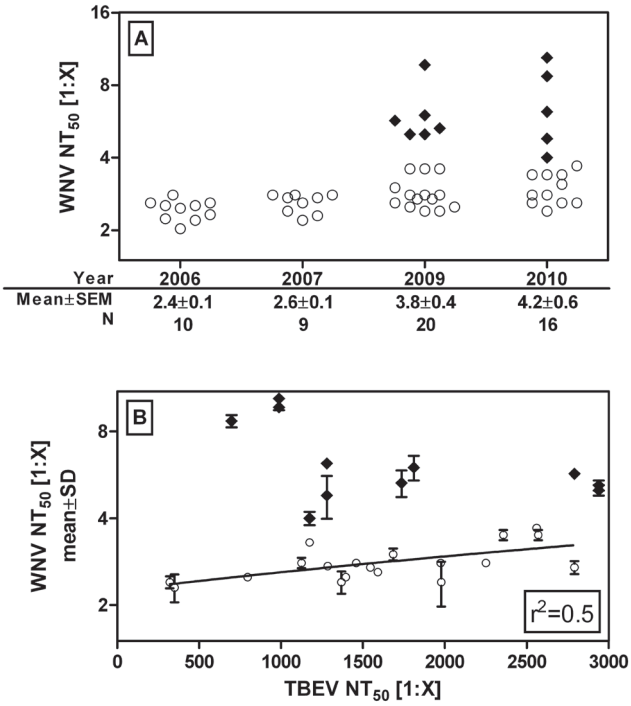
Cases of West Nile virus infection and seropositivity in EU countries, Russia and Israel, by year of occurrence, 1962–2010

Year	Country	Cases	WNND	Fatalities	Seropositivity	CFR [%]	Lineage	Reference
1962–1964	France	13	NI	0	NI	NI	NI	[4]
1973	Portugal	NI	NI	NI	0.5% (1,649 sera anti-WNV-positive)	NI	NI	[5]
1973	Spain	NI	NI	NI	17% (701 sera anti-Flavivirus-positive)	NI	NI	[6]
1975–1979	France	NI	NI	NI	5% (235 sera anti-WNV-positive)	NI	NI	[7]
1975–1976	Spain	NI	NI	NI	8% (1,037 sera anti-WNV-positive)	NI	NI	[8]
1980	Spain	NI	NI	NI	8% (130 sera anti-Flav-positive)	NI	NI	[9]
1982	France	1	1	NI	NI	NI	NI	[10]
1996	Romania	393	352	17	NI	4	1	[11] [12] [13] [14]
1997	Czech Republic	5	0	0	2.1% (619 sera anti-WNV-positive)	NI	NI	[15]
1997–1998	Romania	NI	13	1	4% (959 sera anti-WNV-positive)	NI	NI	[12] [16]
1999	Czech Republic	4	NI	NI	NI	NI	NI	[15]
1999	Czech Republic	NI	NI	NI	2% (619 sera anti-WNV-positive)	NI	NI	[15]
1999	Russia	318	84	40	NI	13	1	[11] [17] [18]
2000	Russia	56	20	0	NI	NI	NI	[12] [17]
2000	Israel	417	307	35	NI	8	NI	[19] [20]
2001	Russia	64	NI	NI	NI	5-10	NI	[12] [17]
2002	Czech Republic	1	0	0	NI	NI	NI	[21]
2002	Spain	NI	NI	NI	1% (797 sera anti-Flavivirus-positive)	NI	NI	[22]
2003	France	4	2	0	NI	NI	NI	[23]
2003	Hungary	NI	14	0	NI	NI	NI	[24]
2007	Greece	NI	NI	NI	1.02% (4 sera anti-WNV-positive)	NI	NI	[2]
2007	Russia	54	NI	2	NI	4	2	[25]
2008	Hungary	NI	14	0	NI	NI	NI	[26]
2008	Italy	13	8	0	NI	NI	NI	[27] [28]
2009	Italy	NI	17	3	NI	NI	1	[29] [30]
2010	Hungary	3	NI	NI	NI	NI	NI	[31]
2010	Russia	448	26	6	NI	1	NI	[25] [32]
2010	Romania	41	NI	4	NI	10	NI	[25] [31]
2010	Greece	261	191	35	1.5% (392 sera anti-WNV-positive)	18	2	[31] [33] [34]

CFR: case fatality rate; NI: no information available; WNND: case with West Nile neuroinvasive disease; WNV: West Nile virus.

Figure 1

West Nile virus and tick-borne encephalitis virus neutralisation by intravenous immunoglobulin lots produced from plasma collected in Austria, Germany and the Czech Republic (N=55)



IVIG: intravenous immunoglobulins; NT_{50}: the reciprocal dilution resulting in 50% virus neutralisation; TBEV: tick-borne encephalitis virus; SD: standard deviation; SEM: standard error of the means; WNV: West Nile virus.

A: WNV neutralisation titres determined in IVIG lots produced between 2006 and 2010. WNV neutralisation titres within (open circles) and out of (black diamonds) the correlation slope as shown in B. Oneway ANOVA analysis revealed a systematic (p=0.0269) mean increase in NT_{50} of IVIG sorted by production year.
B: Correlation analysis (r^2=0.5, p=0.002) of EU plasma-derived IVIG lots (N=30) tested at least in duplicate for WNV and TBEV neutralisation. Results are given as mean NT_{50}±SD.

IVIG lots exclusively collected from EU plasma were not available for the year 2008.

by approximately 6–8 months. The IVIG lots containing significantly higher WNV-neutralising capacity (Figure 1, black diamonds) did not follow a seasonal pattern.

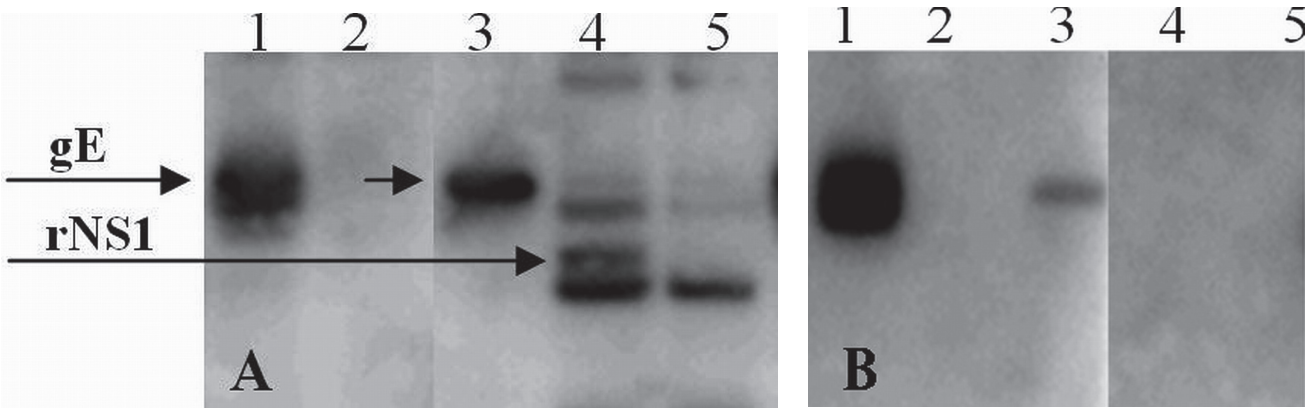
The non-structural flavivirus protein NS1 is only expressed during infection, but not present in the inactivated whole virus vaccines that are used in the countries analysed here. In addition, reactivity of antibodies with the NS1 protein is specific to the flavivirus serotype, and thus infections with either WNV or TBEV can be differentiated [36]. We therefore analysed the antibody specificity of six randomly selected IVIG lots, produced in 2007 (n=1) and 2009 (n=5), by Western blot. TBEV- or WNV-infected Vero cells as well as recombinant WNV NS1 antigen were used as a positive and uninfected Vero cells as a negative control [37, 38]. The blots were incubated with IVIG produced from plasma collected in Austria, Germany and the Czech Republic (Figure 2A), or with a control serum from TBEV-infected mice (Figure 2B). IVIG interacted with the flavivirus structural envelope protein E as well as the WNV-specific NS1 (Figure 2A). In contrast, the control mouse serum reacted strongly only with the E protein of TBEV-infected cells and weakly with WNV-infected cells (Figure 2B).

Discussion and conclusion

We found that IVIG preparations manufactured from plasma collected in Austria, Germany and the Czech Republic, contained neutralising antibodies against WNV at titres which have increased significantly since 2009. As WNV and TBEV are related flaviviruses, albeit distantly, we quantified neutralising antibody titres against TBEV in these IVIG lots, to investigate cross-reactivity between these two viruses as a potentially confounding variable. Indeed, very high TBEV NT_{50} titres of between 400 and 3,000 were observed in all

Figure 2

Reactivity of intravenous immunoglobulin lots produced from plasma collected in Austria, Germany and the Czech Republic with viral proteins



HRP: horseradish peroxidise; IVIG: intravenous immunoglobulins; NT_{50}: the reciprocal dilution resulting in 50% virus neutralisation; TBEV: tick-borne encephalitis virus; PVDF: polyvinylidene fluoride; SD: standard deviation; SDS PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; WNV: West Nile virus.

SDS PAGE transferred to Immobilon PVDF membranes and probed with (A) EU IVIG (1:10; WNV NT_{50} mean±SD: 1.6±1.2, n=4) and HRP-coupled anti-human IgG (1:10,000) or (B) with TBEV-infected mouse serum (1:200) and HRP-conjugated anti-mouse IgG (1:10,000).

Lanes 1: Supernatants of TBEV-infected Vero cells; lanes 2: uninfected Vero cells (negative control); lanes 3: WNV-infected Vero cells, lanes 4: *E.coli*-expressed recombinant NS1 (positive control).

The figure shows one representative result of six IVIG lots tested.

investigated years, a result of vaccination and possibly subclinical TBEV infections. Despite a significant correlation of TBEV and WNV neutralisation titres in approximately 60% of the IVIG lots, 11 of the 30 lots contained significantly higher ($p<0.0001$) neutralisation titres against WNV (mean±standard error of the means (SEM): 6.5±0.6, n=11, compared with 2.8±0.1, n=19) that did not correlate with the TBEV-neutralising capacity. IVIG lots produced from plasma collected in Austria, Germany and the Czech Republic were shown by Western Blot to contain specific antibodies against WNV NS1, the most useful differentiation marker for flavivirus infections in humans [36], which provided further evidence for past WNV infections in plasma donors from the central part of the EU. As the detection of antibodies to the WNV NS1 protein is serotype-specific, a possible contribution of antibodies against dengue virus can be excluded. The theoretical possibility of a contribution of neutralising antibodies to Usutu virus (USUV) [39], a virus that belongs to the same serocomplex as WNV, was not evaluated in vitro, as even the highest USUV activity in Austria as observed during the summer of 2003 [40] had no impact on the WNV-specific neutralisation titres in IVIG lots [41]. Moreover, despite a comprehensive surveillance programme for dead birds and mosquitoes in Austria [42], where around 45% of the plasma is collected for production of the IVIG lots tested in this study, no evidence of human exposure to USUV could be found [43], which makes a significant contribution of USUV antibodies to WNV neutralisation titres determined in the present study unlikely. However, additional studies would be relevant to describe this issue more precisely.

These results demonstrate WNV seropositivity in asymptomatic plasma donors from Austria, Germany and the Czech Republic, although some of the seropositivity could be due to travel-related infections. The present epidemiological situation in these countries is thus similar to the one in Greece before the recent epidemic. The causative agent of the outbreak in Greece, WNV lineage 2, has only once before been isolated from humans with severe clinical progression, in an outbreak in Volgograd, Russia in 2007 [18]. Before those two outbreaks, this lineage was considered to be less virulent in humans compared to isolates belonging to the lineage 1 [44]. It has been suggested that the evolution from low to high human pathogenicity is associated with mutations of only a few amino acids, most likely in the non structural proteins [45].

The WNV neutralisation capacity of IVIG lots produced from Austria, Germany and the Czech Republic has increased from a mean NT_{50} of 2.5 in 2006 and 2007 to 4.2 at the beginning of 2010, which accounts for an increase of 1.7 in the WNV neutralising capacity (Figure 1). The TBEV neutralisation titres of IVIG have not changed over time, and thus the increase in WNV neutralisation titres is most likely a true reflection of increased virus circulation. Using the same, fully validated assay, reconvalescent sera from human WNV cases in North America have earlier been shown to have a mean NT_{50} of 208 [41], 120-fold higher than the increase now observed. This would indicate past WNV exposure of just under 1% of the population of Austria, Germany and the Czech Republic. The increasing WNV seropositivity in these countries marks this virus as a potential public health concern in this area, and a future epidemic associated with human morbidity and mortality similar to that observed in summer 2010 in Greece cannot be excluded.

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Conflict of interest
The authors of this manuscript have conflicts of interest to disclose: All authors were employees of Baxter AG and the work reported in this article was funded by Baxter AG.

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Prevalence of IgM and IgG antibodies to West Nile virus among blood donors in an affected area of north-eastern Italy, summer 2009

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Following reports of West Nile neuroinvasive disease in the north-eastern area of Italy in 2009, all blood donations dating from the period between 1 August and 31 October 2009 in the Rovigo province of the Veneto region were routinely checked to exclude those with a positive nucleic acid test for West Nile virus (WNV). Only one of 5,726 blood donations was positive (17.5 per 100,000 donations; 95% confidence interval (CI): 0.4–97.3). In addition, a selection of 2,507 blood donations collected during the period from 20 July to 15 November 2009 were screened by ELISA for IgG and IgM antibodies against WNV. A positive result was received for 94 of them. The positive sera were further evaluated using immunofluorescence and plaque reduction neutralisation test (PRNT), in which only 17 sera were confirmed positive. This corresponds to a prevalence of 6.8 per 1,000 sera (95% CI: 4.0–10.9). In a case-control study that matched each of the 17 PRNT-positive sera with four negative sera with the same date of donation and same donation centre, we did not find a significant association with age and sex of the donor; donors who worked mainly outdoors were significantly more at risk to have a positive PRNT for WNV.

Introduction

West Nile virus (WNV) was originally identified in 1937 in northern Uganda [1]. Birds, especially those in the *Corvidae* family are the natural reservoir of the virus, which is mainly transmitted to humans through the bite of infected *Culex* mosquitoes [2,3]. Transmission through receipt of blood products and transplantation has also been documented [4,5]. Rare cases of vertical transmission (i.e. transplacentally or through breast milk) and laboratory-acquired infection have also been reported [6].

During the last 20 years, several WNV outbreaks have occurred throughout the world. In addition to the United States [7], where the virus was first identified in 1999 [2,3,6], large WNV outbreaks have also been reported in Europe and in the Mediterranean basin [8,9]. The largest European outbreak occurred in 1996 in Bucharest, Romania [10]. In the Mediterranean area, the first outbreak of human encephalitis was identified in the Camargue, France, in 1962 [11], followed by other epidemics in the 1990s involving a relatively high number of human cases in Algeria [8], Tunisia [12] and Israel [13,14]. In the past few years, cases of WNV encephalitis have also been reported in the Volgograd region in Russia [15], in Hungary [16] and Romania [17], and very recently in Greece [18].

In Italy, the first outbreak of WNV infection was reported in 1998 in horses in Tuscany [19]. The virus re-emerged in north-eastern Italy in summer 2008, when equine cases of WNV neuroinvasive infection were notified in the regions Veneto and Emilia Romagna [20]. An extraordinary WNV surveillance programme was subsequently activated, which led to the notification of nine human cases of West Nile neuroinvasive disease (WNND) in the summer of 2008 [21,22] and a further 16 cases in late summer 2009; all cases occurred in the regions Emilia Romagna, Veneto and Lombardia, in wet areas surrounding the Po river [23-25].

Since WNV infection is generally asymptomatic with encephalitis occurring in less than 1% of cases, we conducted a seroepidemiology investigation in the Rovigo province in northern Italy, where a high incidence of WNND was observed, in order to estimate the extent of the epidemic and to better plan intervention strategies.

Methods

Setting

The study area was the province of Rovigo, Veneto region, north-east Italy. This province, which has around 250,000 inhabitants, borders with the Po river and the Emilia-Romagna region. All territory is a level land characterised by extraordinary biodiversity, mainly because of the presence of freshwater and brackish water wetlands, including flooded deciduous woodlands, open lagoons of shallow water and river mouths. The high humidity level makes this area particularly attractive for mosquitoes, in particular during summer. It is located at the crossroads of bird migration routes connecting Europe, the Mediterranean basin and Africa, hosting a high number and wide range of migrating birds throughout the year. Thus, the high concentration of mosquitoes and migrating birds creates opportunities for vector-borne viruses such as WNV [26].

Study design

The study involved the three blood donation centres of Rovigo province which collected about 18,500 blood donations in 2009. During the period from 1 August to 31 October 2009, all blood donations were routinely evaluated by nucleic acid amplification test (NAAT) for WNV to identify potentially viraemic donations. In order to study the prevalence of WNV in the area, we tested, during the period from 20 July to 15 November 2009, serum samples from 25 blood donations per day for IgG and IgM antibodies to WNV. Any IgM- or IgG-positive sample was further evaluated by immunofluorescence and by plaque-reduction neutralisation test (PRNT) for confirmation. The number of serum samples collected at each centre was proportional to the volume of donations performed in the year 2008. Thus, each day, five donations were sampled from Adria (Centre 1), five from Trecenta (Centre 2), and 15 from Rovigo (Centre 3), choosing serum samples from the first consecutive daily donors who gave their consent to the study. All samples were handled anonymously by technicians and researchers involved in this study.

Table 1

Serological results of serum samples positive in the West Nile virus ELISA screening, Rovigo province, Italy, 20 July–15 November 2009 (n=94)

IgG ELISA	IgM ELISA	IgG IFA	IgM IFA	Number of samples
Confirmed positive by PRNT				17
+	+	+	+	7
+	-	+	-	9
+	+	+	-	1
Not confirmed positive by PRNT				77
+	-	+	-	46
+	-	+	+	3
+	-	-	-	19
+	+	+	-	1
-	+	+	+	2
-	+	-	+	1
-	+	-	-	5

ELISA: enzyme-linked immunosorbent assay; IFA: immunofluorescence assay; PRNT: plaque reduction neutralisation test.

To evaluate potential risk factors associated with WNV infection, a case–control study matched by day and donation centre was performed. More specifically, for all donors that had IgG and/or IgM to WNV confirmed by PRNT, specific information about age, sex, address and type of job was retrospectively collected. For each positive donor, we collected the same information from four negative cases who were randomly chosen among the donors seen on the same day in the same centre. Although details on the type of job were collected, we decided after preliminary analysis to create a dummy variable classifying the job as done predominantly in the open air or not, e.g. a builder was classified as an open-air worker while a bank clerk's work was classified as indoors.

Laboratory testing

WNV NAAT screening was performed using Cobas Taq Screen West Nile Virus test on a Cobas s201 system (Roche Molecular Systems) on pools of aliquots from six individual plasma specimens. Specimens included in WNV RNA-positive pools were re-tested individually with the same WNV NAAT kit.

WNV IgM and IgG testing was done using the WNV IgM capture DxSelect ELISA and IgG DxSelect ELISA kits (Focus Diagnostics), respectively, as reported [18]. All serum samples which tested positive in the ELISA were further analysed by anti-West Nile virus IIFT IgG and IgM immunofluorescence assays (IFA) (Euroimmun AG), and, to rule out cross-reactivity with other flaviviruses and confirm the result, also with the PRNT, according to the previously described protocol [18].

Statistical analysis

Prevalence of antibodies to WNV was calculated as the ratio between sera confirmed positive by PRNT and all tested sera. The 95% confidence intervals (CI) of the prevalence were calculated using the binomial distribution. Prevalence estimates were also stratified by blood donation centre and by month of donation. Chi-square test was used to evaluate if the prevalence by blood donation centre and by month of donation was statistically significant.

Sensitivity, specificity, positive predictive (PPV) and negative predictive value (NPV) of IgG and IgM with respect to PRNT were also calculated, assuming that all sera not evaluated by PRNT were negative. This assumption favours highest estimates for specificity and NPV.

Odds ratios (OR) were calculated to evaluate the association between age, sex and open-air/indoors job of the donor with confirmed positivity by PRNT compared

Table 2

Sensitivity, specificity, negative and positive predictive value of serological tests for West Nile virus, compared with PRNT, Rovigo province, Italy, 20 July to 15 November 2009 (n=2,507)

	PRNT-positive	PRNT-negative	PPV	NPV	Sensitivity	Specificity
WNV ELISA						
IgM-positive/IgG-positive	8	1	88.9	99.6	47.1	100.0
other	9	2,489				
IgM-positive/IgG-negative	0	8	0.0	99.3	0.0	99.7
other	17	2,482				
IgM-negative/IgG-positive	9	68	11.7	99.7	52.9	97.3
other	8	2,422				
ELISA-positive	17	77	18.1	100.0	100.0	96.9
ELISA-negative	0	2,413				
WNV IFA [a]						
IgM-positive/IgG-positive	7	5	58.3	99.6	41.2	99.8
Other	10	2,485				
IgM-positive/IgG-negative	0	1	0.0	99.3	0.0	100.0
other	17	2,489				
IgM-negative/IgG-positive	10	47	17.5	99.7	58.8	98.1
other	7	2,443				
IFA-positive	17	53	24.3	100.0	100.0	97.9
IFA-negative	0	2,437				

ELISA: enzyme-linked immunosorbent assay; IFA: immunofluorescence assay; NPV: negative predictive value; PPV: positive predictive value; PRNT: plaque reduction neutralisation test.

[a] WNV IFA is evaluated as a second line test for WNV ELISA-positive samples.

Note: It was assumed that all sera tested negative by ELISA would also have been negative in IFA and PRNT even when these tests were not performed.

Table 3

Adjusted odds ratios of being PRNT-positive for West Nile virus, associated with blood donor characteristics, conditional logistic model, Rovigo province, Italy, 20 July–15 November 2009 (n=17)

		PRNT-positive		PRNT-negative[a]		AOR	95% CI	p
		N	%	N	%			
Age (years)	‹40	5	23,8	16	76,2	1.00		
	40-46	4	18,2	18	81,8	0.42	0.08-2.14	0.30
	47-53	4	19,0	17	81,0	0.48	0.09-2.45	0.37
	›53	4	19,0	17		0.69	0.15-3.23	0.64
Sex	Male	16	20,5	62	79,5	1.00		
	Female	1	14,3	6	85,7	0.88	0.09-8.75	0.92
Indoor/outdoor working activity	Indoors	5	16,2	57	83,8	1.00		
	Outdoors	11	38,5	8	61,5	5.07	1.01-25.37	0.05
	Unknown	1	25.0	3	75.0	2.74	0.20-37.38	0.45

AOR: adjusted odds ratios; CI: confidence interval; PRNT: plaque reduction neutralisation test.

[a] PRNT-negative blood donors were matched to positive ones by day and centre of donation.

to matched sera not tested/not confirmed by PRNT. The OR was calculated taking into account matching by day and blood donation centre. A conditional logistic model was used to simultaneously adjust the OR for the effect of each evaluated factor with respect to the others.

Results

During the period from 1 August to 31 October 2009, 5,726 blood donations were collected in Rovigo province and tested by WNV NAAT. One blood donation, not included in the seroprevalence study, was WNV RNA-positive (17.5 per 100,000 donations; 95% CI: 0.4–97.3). Details on this positive case have been reported previously [25].

Among the 2,507 serum samples evaluated for IgG and IgM, 94 (3.7%) were positive in the WNV ELISA IgG and/or IgM screening and further tested by IFA and PRNT (Table 1). Of the 94 ELISA-positive samples, 70 (75%) were also WNV IFA-positive and 17 (18%) were confirmed by PRNT. The PRNT-confirmed cases included eight (47%) cases with both WNV IgM and IgG and nine (53%) with only WNV IgG detected by ELISA and/or IFA.

The estimated overall prevalence of WNV antibodies confirmed by PRNT was 6.8 per 1,000 tested sera (95% CI: 4.0–10.9). Stratifying by month of donation no particular fluctuations were observed, with prevalences that varied from 6.2 per 1,000 in September to 7.6 in October (p=0.99, Chi-square test, data not shown). The prevalence differed significantly by blood donation centre, with the highest prevalence in Trecenta (17.9 per 1,000 donations) and the lowest in Adria and Rovigo (4.0 per 1,000 donations) ($p<0.01$).

Table 2 shows the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of WNV ELISA alone and WNV ELISA followed by WNV IFA as a second-line test compared with PRNT results as the gold standard, and assuming that all ELISA-negative samples that were not also evaluated by PRNT, would have been negative in the PRNT. The sensitivity of WNV IgM detection for predicting WNV infection was low, whether by ELISA alone or by a combination of ELISA and IFA; however, the possibility of false negative results of PRNT at early phases of infection was not excluded in this study. The sensitivity of WNV IgG detection, either alone or in combination with WNV IgM detection by ELISA was about 50%. The addition of WNV IFA as a second-line test for ELISA-positive samples did not improve test sensitivity and PPV.

Table 3 shows the association of the blood donors' characteristics with being WNV positive in PRNT in a matched case-control analysis. While age and sex were not found to be significantly associated with being WNV-positive in PRNT, working outdoors was associated with a statistically significant higher risk (>5 times) of WNV infection than working indoors (p=0.05).

Finally, a map of the province was produced, plotting the geographical coordinates of the PRNT-positive donors and the cases of WNND notified in 2009 and resident in the Rovigo province. Most subjects with a PRNT-confirmed WNV infection lived close to rivers in western areas of the Rovigo province, without any apparent clustering or association with the place of residence of subjects diagnosed with WNND (data not shown).

Discussion

In accordance with national guidelines [27] that recommend screening of blood donations in affected areas where at least one human case with WNND has been detected, all blood donations performed in Rovigo province during summer 2009 were individually tested by NAAT. Only one of 5,726 blood donations resulted positive by NAAT, which corresponds to an estimated risk of WNV transmission of about 17.5 per 100,000 blood donations. With the limitation of the small samples size in our study, this risk appears to be lower than that reported in the United States during the peak of the WNV epidemic in 2002 [28] and in Canada in the period from 2006 to 2007 [29].

In order to estimate the extent of WNV infection among humans, we also conducted a serosurvey in a selection of blood donations in the study area; the estimated overall prevalence of anti-WNV antibodies was 6.8 per 1,000 sera (95% CI: 4.0–10.9). This prevalence estimate was lower than that found in a previous survey (15.6 per 1,000), that was conducted in the same province but was restricted to a population considered at risk of environmental exposure represented by farm employees who worked in areas where WNV-seropositive horses had been identified [22].

A retrospective screening of solid organ donors in several Italian regions found a higher prevalence of anti-WNV antibodies than the present study on blood donors [30]. This discrepancy could be accounted for by differences in target populations and sampling strategies as well as laboratory methods used for testing and for confirmation of positive results. We considered as positive only those samples which were confirmed by neutralisation assays. In fact, less than 20% of WNV ELISA-positive samples were confirmed by PRNT. After combining ELISA and IFA, the specificity did not increase significantly for those samples which were positive in both tests. Since the PPV of a test tends to decrease in low prevalence areas, our results suggest that neutralisation assays should be used to confirm positive results, especially in areas with a low risk of infection.

The prevalence in our study varied widely among geographical areas, ranging from about 18 per 1,000 blood donations in Trecenta to 4.0 per 1,000 in Adria and Rovigo. Moreover, the geographical distribution of infected individuals suggested that most cases were resident in areas near rivers and other water sources.

This is consistent with other studies on WNV and other mosquito-borne infections, such as La Crosse encephalitis [3,31]. However, we could not evaluate the association between WNV seroprevalence and proximity to water sources in our samples because of the lack of available geo-reference data on WNV-negative blood donations. For the same reason, any factor explaining the geographical variation could not be evaluated due to limited environmental data.

With regard to risk factors for infection, no statistically significant difference was found for age or sex, while a statistically significant association was found with working outdoors compared with other jobs. These findings, which suggest a higher risk of exposure to mosquito bites, contradict studies conducted in Romania, where having spent more than six hours outdoors during the day was not found associated with West Nile virus infection [32].

In conclusion, this study estimated the seroprevalence of WNV among blood donors of an affected area of Italy as 6.8 per 1,000 sera and indicates that WNV seroprevalence can vary widely even between different geographical areas within the same province. Although WNV NAT-positive samples were rare in our population, blood screening is needed in order to reduce the risk of WNV transmission to vulnerable recipients.

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Geographical clustering of cases of infection with moxifloxacin-resistant *Clostridium difficile* PCR-ribotypes 012, 017 and 046 in Sweden, 2008 and 2009

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We report the results of two nationwide surveillance studies of *Clostridium difficile* infection conducted during 2008 and 2009 in Sweden. The first study aimed to identify and quantify the proportion of *C. difficile* isolates with decreased susceptibility to moxifloxacin, particularly those of PCR-ribotype 027. From December 2007 to September 2008, 20 of 28 regional laboratories sent 585 isolates to the Swedish Institute for Infectious Disease Control for typing. A majority of the isolates (454 of 585; 78%) belonged to four PCR ribotypes (012, SE37, 017 and 046), all clustered in geographical regions. Only two type 027 isolates were found, both from the same patient. In the second study, involving all 28 regional laboratories, all consecutive *C. difficile* isolates collected during two time periods in 2009 (n=364) were typed and tested for susceptibility to clindamycin, erythromycin, moxifloxacin, metronidazole and vancomycin. The three most common PCR ribotypes were SE21, 001 and 020 (22% of all isolates). Types 012, 017, and 046 were geographically clustered and associated with decreased susceptibility to moxifloxacin, clindamycin and erythromcin. The extent of moxifloxacin prescription was highly variable among counties, indicating a need for careful monitoring of prescription rates to follow its role in *C. difficile* epidemiology.

Introduction

Clostridium difficile infection is a common nosocomial problem in elderly patients following antibiotic treatment [1]. *C. difficile* is typically isolated from faeces of 1–5% of healthy individuals, but colonisation increases upon admission to hospitals. Hospital-based studies performed in 1999 to 2001 showed that nosocomial spread of *C. difficile* was rare in Swedish hospitals in which infection control routines were implemented, although there were small outbreaks associated with multiresistant PCR-ribotype 012/SE17 [2-4]. Many outbreaks involving other *C. difficile* types have been reported in Europe and the rest of the world [5-8], particularly those caused by moxifloxacin-resistant PCR-ribotype 027/PFGE NAP1 [9-17]. The expansion of certain *C. difficile* types during 2004 to 2008 prompted us to initiate a broader nationwide *C. difficile* surveillance strategy in Sweden. First, in an aim to find virulent type 027, a screening of *C. difficile* isolates with reduced susceptibility to moxifloxacin was performed during 2008. Second, a surveillance programme, including epidemiological typing and antibiotic susceptibility testing, was launched in 2009. Third, a web-based system for continuous voluntary laboratory reporting was initiated in 2009. This article presents the epidemiological situation of *C. difficile* in Sweden in 2008 and 2009 with respect to the occurrence of various PCR ribotypes and their antibiotic susceptibility.

Methods

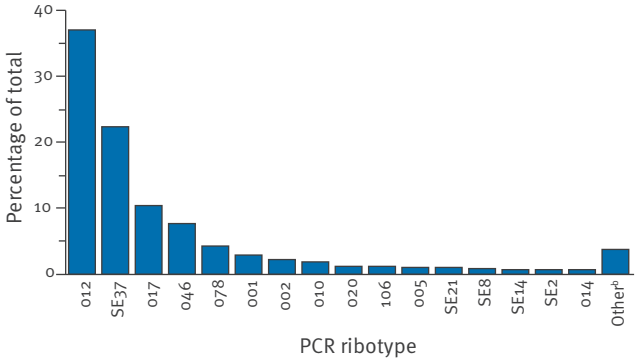
Screening of *C. difficile* isolates with decreased susceptibility to moxifloxacin (2008)

An invitation to participate in the study, including instructions, methods and study protocol, was sent to all 28 regional clinical laboratories in Sweden. Faecal specimens sent by the clinicians for routine *C. difficile* detection were tested in the regional laboratories according to their standard methodology (ELISA/EIA was the most commonly used method, in 24 of the 28 laboratories, followed by cell cytotoxicity assay). Sample material that was positive for *C. difficile* was inoculated anaerobically on taurocholate-cefoxitin-cycloserine-fructose agar (TCCFA) for 48 hours and toxigenic *C. difficile* colonies were subcultured on blood agar. Susceptibility to moxifloxacin was tested using disc diffusion (5 µg) on IsoSensitest agar (Oxoid, United Kingdom) supplemented with 5% defibrinated horse blood and 20 µg/ml beta-NAD incubated at 36 °C for 48 hours. *C. difficile* strains with an inhibition zone diameter ›16 mm (ATCC9689) and 6 mm (Ö07-1702) were used as controls. All *C. difficile* isolates with zone diameter ‹16 mm were sent to the Swedish Institute for Infectious Disease Control for typing. A protocol with

strain number, diameter of the inhibition zone, and, if possible, the total number of screened isolates were included each shipment. Upon arrival, samples were inoculated on both TCCFA and blood agar and incubated anaerobically at 36 °C for 48 hours. Isolated strains were verified for susceptibility to moxifloxacin using the disc diffusion method described above and Etest (AB Biodisk, Sweden). Of 641 sent samples, 37 were excluded because *C. difficile* could not be isolated and/or the sample was contaminated; a further 19 isolates were omitted due to an inhibition zone diameter >16 mm, giving a final 585 *C. difficile* isolates. The study period was from 7 December 2007 to 1 September 2008. Since no patient data were included, we could not judge how many patients or how many cases of *C. difficile* infection there were during the study period.

Figure 1

Proportion of *Clostridium difficile* PCR ribotypes[a] with decreased susceptibility to moxifloxacin, Sweden, 2008 (n=585)



[a] The three-digit international nomenclature is used for typing, or when reference isolates are missing, SE-type is used.

[b] Ribotypes represented by fewer than three isolates per type.

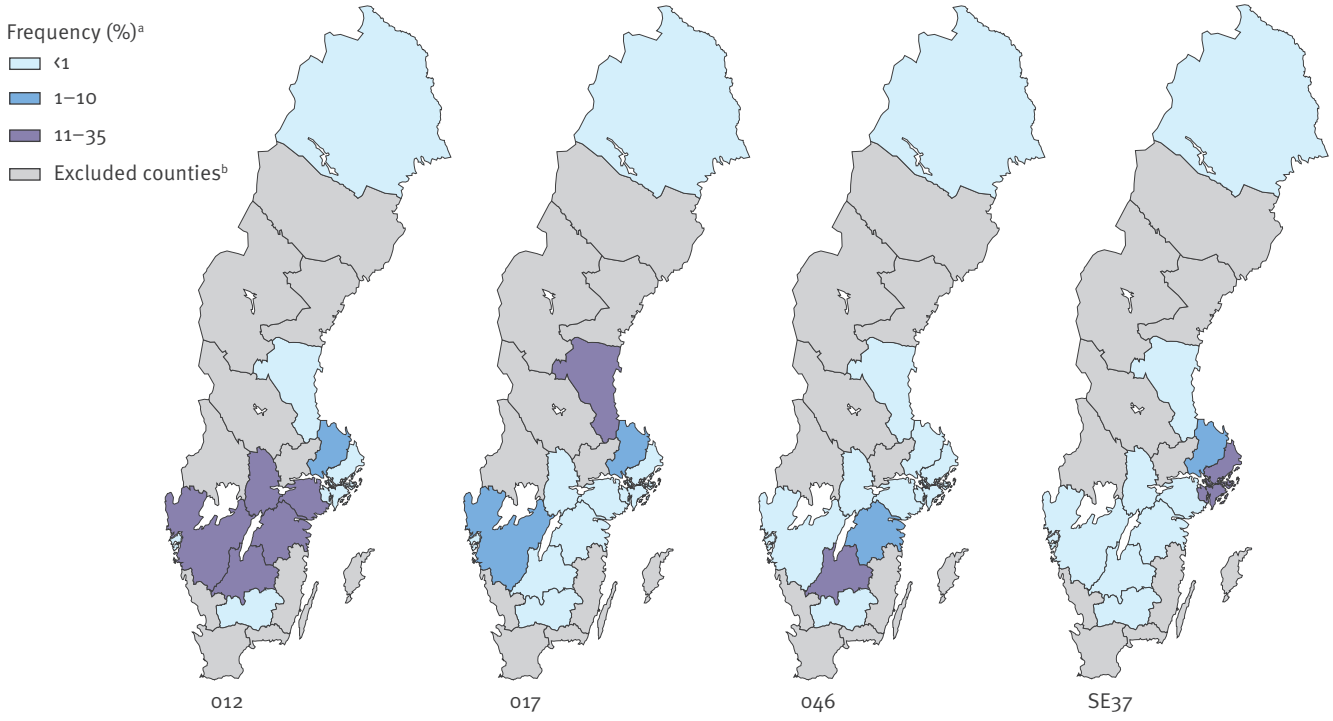
National surveillance typing (2009)

An invitation to participate in the surveillance programme, including methods and study protocol, was sent to all 28 regional Swedish laboratories. All consecutive strains isolated from *C. difficile*-positive samples obtained through routine detection during 9–15 April and 21–27 September 2009 were sent to the Swedish Institute for Infectious Disease Control for typing and susceptibility testing for moxifloxacin, erythromycin, clindamycin, metronidazole and vancomycin. Of 432 samples sent, 40 were excluded because they were contaminated or not viable. Of the remaining 392 isolates, 28 were not analysed because their dates of isolation did not comply with the inclusion criteria above, giving a final total of 364 isolates.

Susceptibility testing was performed using Etest on Mueller-Hinton agar medium (BD Diagnostics) at 35°C for 48 hours. A bacterial density of McFarland 1 was used to obtain a next-to-confluent layer of *C. difficile* bacteria. There were no significant differences in minimum inhibitory concentration (MIC) values using Mueller-Hinton or IsoSensitest agar (data

Figure 2

Geographical distribution of dominating *Clostridium difficile* PCR ribotypes with decreased susceptibility to moxifloxacin, Sweden, 2008 (n=454)



[a] The frequency is calculated as the number of isolates of the indicated type divided by the total number of screened isolates in each county multiplied by 100.

[b] Due to missing numbers of total screened isolates during the study period from some laboratories, calculation of frequency was only possible for 10 of the 21 counties.

not shown). Breakpoints were chosen according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) tentative ecological cut-off for detection of isolates with resistance mechanisms, as suggested by the European Study Group for *C. difficile* [18].

PCR ribotyping

PCR ribotyping was performed according to the method of Stubbs et al. [8], with modifications described in Svenungsson et al. [4]. Reference isolates were obtained from the Cardiff-European Centre for Disease Prevention and Control (ECDC) collection of *C. difficile* strains (obtained from Ed J. Kuijper, Leiden University Center, Leiden, the Netherlands). Bionumerics software version 6.1 (Applied Maths NV, Sint-Martens-Latem, Belgium) was used for the analysis. The three-digit international nomenclature was used for typing or, when reference isolates were missing, an SE-type was given.

Prescription of antibiotics

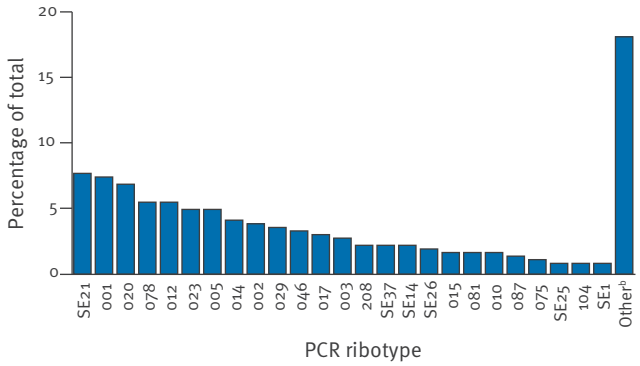
Sales data of antibiotics were obtained from Apotekens Service AB, which is responsible for compilation of all drug statistics, including sales, in Sweden. The data include antibiotics prescribed to patients as well as sales to hospitals and other healthcare providers.

Statistical methods

Fisher's exact test and chi-square tests were performed to examine differences in geographical distribution of ribotypes using R v. 2.10.1. Kaleidagraph v. 4.02 (Synergy software) was used for data management and graphics. To avoid type-I error due to multiple statistical tests, a significance threshold of p=0.005 was applied.

Figure 3

Proportion of *Clostridium difficile* PCR ribotypes[a], Sweden, 2009 (n=364)



[a] The three-digit international nomenclature is used for typing, or when reference isolates are missing, SE-type is used.

[b] Ribotypes represented by fewer than three isolates per type.

Results

C. difficile isolates with decreased susceptibility against moxifloxacin (2008)

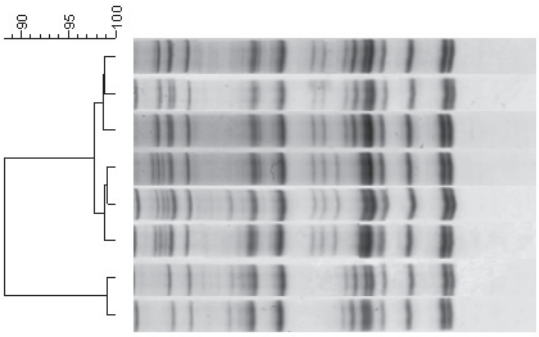
A total of 20 of the 28 Swedish regional laboratories covering 15 of the 21 of the counties supplied a total of 585 isolates with reduced susceptibility to moxifloxacin. Of the 15 counties, 10 supplied data on the number of screened isolates: 546 of 2,702 isolates (20%; range: 9–34%) had decreased susceptibility to moxifloxacin. Of the 585 isolates (from the 15 laboratories), 454 (78%) belonged to four PCR ribotypes: 012, SE37, 017 and 046 (Figure 1). Types 012 and 046 were found mainly in the central and southern part of Sweden, while types 017 and SE37 were predominant in the central-east counties of Gävleborg and Stockholm, respectively (Figure 2, shown in purple). The distributions for each of the types 012, 017, 046, 078 and SE37, compared with that of other types, were highly skewed between counties: a chi-square test among counties having more than 10 isolates available for typing gave a p value of ‹0.001 for each of the types 012, 017, 046, 078 and SE37 (for 9 of the 21 counties). Only two type 027 isolates were found, both from the same patient.

National surveillance typing programme (2009)

Of 364 C. difficile isolates supplied by all 28 regional laboratories, type SE21 was found in highest number followed by types 001, 020, 078, 012 and 023 (Figure

Figure 4

PCR ribotype patterns of the *Clostridium difficile* SE21/014/020 group[a], Sweden, 2009 (n=8)



Strain	PCR ribotype
ATCC 43600	SE21a/014
NT035	SE21a
NT141	SE21a
NT140	SE21b
R10079	SE21b/020
NT094	SE21b
NT020	SE21
NT042	SE21

ECDC: European Centre for Disease Prevention and Control; NT: national typing.

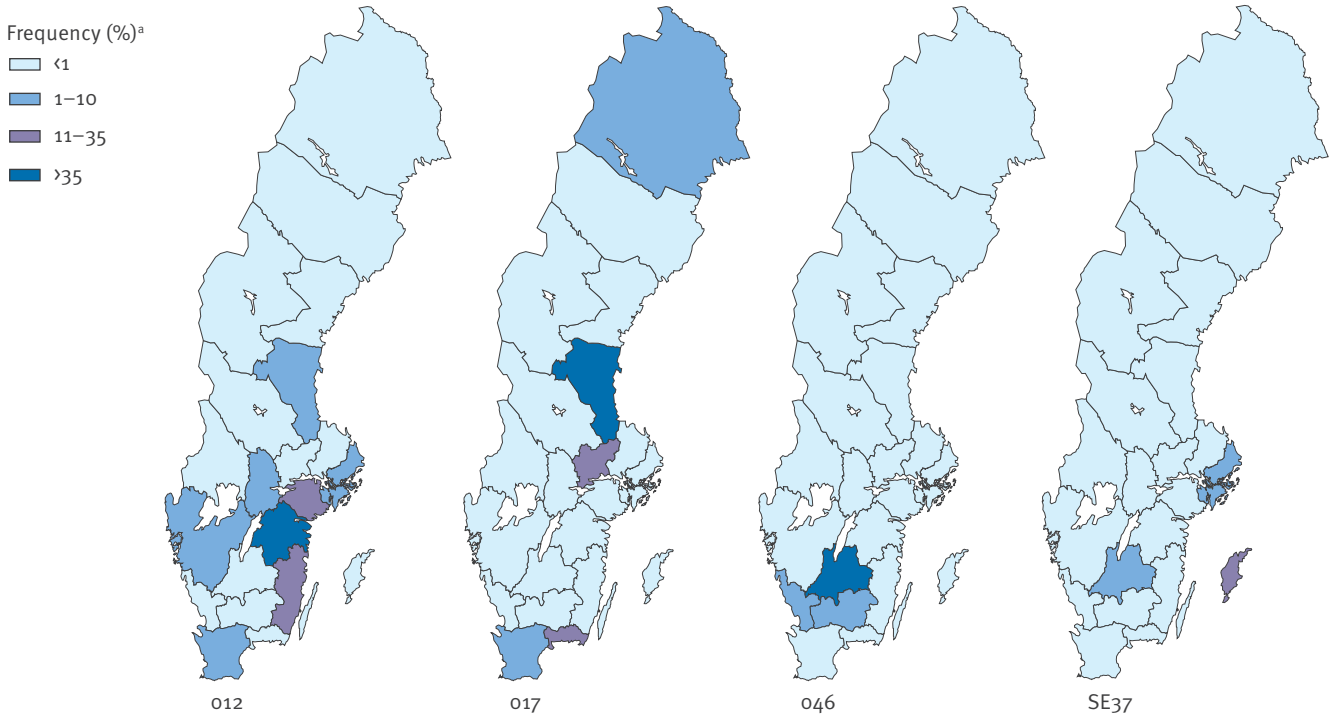
[a] *C. difficile* strains from the 2009 study, designated NT, and the Cardiff-ECDC collection are shown. The dendogram was produced using cluster correlation, Pearson correlation and the unweighted pair group method for arithmetic averages. The scale shows similarity as a percentage.

3). The PCR ribotype pattern of type SE21 was related to those of types SE21a/014 and SE21b/020 (Figure 4): because of their similarities, this group of ribotypes has sometimes been called the '014/020' group (see Discussion). However, in our study we judged these as separate types. Type 012, 017 and 046 were abundant in the same geographical regions as in 2008 and the geographical distributions of these types were significantly asymmetric (Figure 5) (p<0.001 each, Fisher's exact test; all 21 counties). Other types were not clustered or were only weakly so, for example, 001 (p=0.95), 014 (p=0.022), 020 (p=0.012), 023 (p=0.71), 078 (p=0.31), SE21 (p=0.14) and SE37 (p=0.23). One type 027 isolate was detected but it showed a MIC of <4 mg/L against moxifloxacin, which is not characteristic for the epidemic and hypervirulent type 027.

In total, 57 of the 364 (16%) isolates showed decreased susceptibility to moxifloxacin (MIC>32 mg/L); all other isolates displayed MIC values between 0.25 mg/L and 2 mg/L). The geographical distribution of isolates with a MIC>32 mg/L was skewed (p<0.001, Fisher's exact test, n=14, with counties reporting fewer than 10 isolates excluded) and 46 of 57 isolates belonged to type 012, 017, 046 and SE37 (Table). In total, 60 of 364 (16%) isolates showed decreased susceptibility to clindamycin and, again, a majority (46 of 60) isolates belonged to type 012, 017, 046 and SE37 (Table). Of the 364 isolates, 62 (17%) showed decreased susceptibility to

Figure 5

Geographical distribution of *Clostridium difficile* PCR ribotypes associated with decreased moxifloxacin susceptibility in all 21 counties, Sweden, 2009 (n=57)



[a] The frequency is calculated as the number of isolates of the indicated type divided by the total number of screened isolates for each county multiplied by 100.

Table

Proportion of *Clostridium difficile* PCR ribotypes with decreased susceptibility to moxifloxacin, clindamycin and erythromycin, Sweden, 2009 (n=364)

PCR ribotype	Number of isolates tested	Number of isolates with decreased antibiotic susceptibility[a]		
		Moxifloxacin (MIC>4 mg/L)	Clindamycin (MIC>16 mg/L)	Erythromycin (MIC>2 mg/L)
012	20	19	18	15
017	11	10	11	10
046	12	10	10	10
SE37	8	7	7	7
Other	313	11	14	20

MIC: minimum inhibitory concentration.

[a] Breakpoints were chosen according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) tentative ecological cut-off for detection of isolates with resistance mechanisms, as suggested by the European Study Group for *C. difficile*.

erythromycin (Table). No isolate was resistant to metronidazole or vancomycin.

Prescription of moxifloxacin in Sweden in 2008 and 2009

The mean prescription rate of moxifloxacin per county was 0.020 defined daily dose (DDD) per 1,000 inhabitants per day for 2008 and 2009. The prescription rate differed markedly between counties (range: 0–0.088 DDD per 1,000 inhabitants per day) and was highest in the south-east of Sweden (Figure 6). Some of the counties in the south-east also had a high proportion of PCR ribotypes 012 and 046 (Figure 5). However, the prescription rate of moxifloxacin was comparatively low in the counties where types 017 and SE37 dominated.

Discussion and conclusion

PCR ribotyping has been used for studying *C. difficile* epidemiology for several years, and details of the first large *C. difficile* PCR ribotype library were published by Stubbs et al. in 1999 [8]. When the European Centre for Disease Prevention and Control (ECDC) and the Centre for Infectious Disease Control Netherlands initiated a pan-European *C. difficile* infection survey (ECDIS) study in 2008 [19], a *C. difficile* reference strain collection with defined PCR ribotypes was formed (the Cardiff-ECDC collection). One purpose of the collection was to support national laboratories in setting up local typing systems and to aid surveillance studies and international comparisons. We found that a majority of the *C. difficile* strains in Sweden was represented in this reference strain collection. Exceptions were type SE37, for example, which was confirmed in the ECDIS study as one of six novel types (PCR ribotype 231) [19]. Another unknown type was SE21, which closely resembled types SE21a and SE21b as well as reference strains of PCR-ribotypes 014 and 020 [4]. Type SE21 was verified as a member of the 014/020 group: in total this group comprised 19% of all *C. difficile* isolates in our study in Sweden in 2009. The proportion of the 014/020 group among all *C. difficile* isolates was 16% in Europe in 2008 [19].

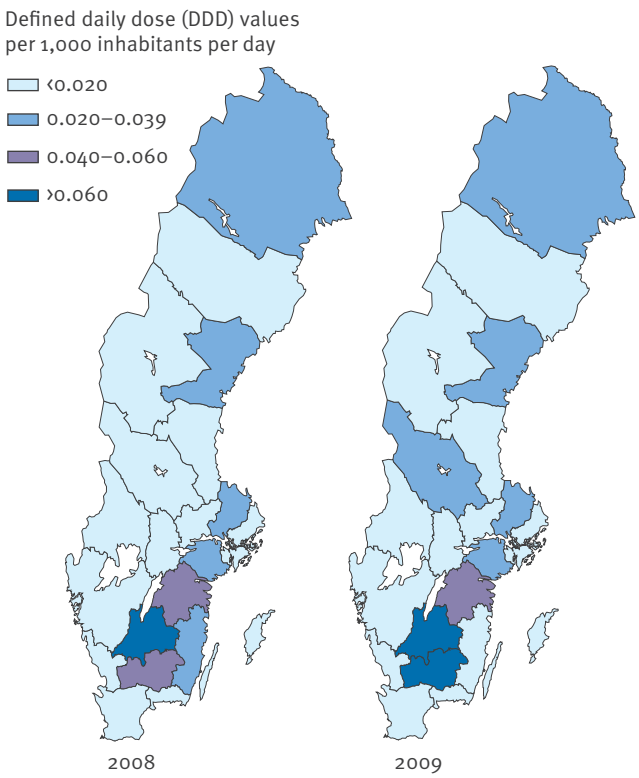
Much interest in recent years has been focused on the worldwide spread of the hypervirulent *C. difficile* type 027 [20,21]. Outbreaks in northern Europe in 2007 to 2009 [10-11,13,22] prompted us to screen for type 027 isolates with decreased susceptibility to moxifloxacin. Of the 585 isolates analysed during 2008, only two type 027 isolates were detected (from the same patient). Furthermore, since no type 027 isolate with reduced susceptibility to moxifloxacin was identified in 2009, such isolates are probably rare in Sweden (compare with [23]). From previous geographically localised studies in Sweden in 1999 to 2001, the six most common types were 014/SE21, 012, 001, 020, 002 and 005 [2-4]. All these were still among the most common types in 2009. There were thus only small changes in the distribution of major types over a period of 10 years, a result quite different from the shift in strain types that has occurred in England [7]. One exception might be type 078, which was the fourth most common type in 2009. Type 078 may be an emerging type in Europe and is commonly found in domestic animals, suggesting transmission through the food chain [24-26].

The studies presented here have several weaknesses. All laboratories did not report a full set of data, particularly from the first screening study. This was mostly due to the fact that several laboratories were in the process of setting up culturing methods and were thus unable to supply isolates from the first day of the study period. Also, the two studies were not of identical design: the first aimed to collect consecutive moxifloxacin-resistant isolates during a longer time period and the second collected all isolates during two shorter time periods. In addition, the short study periods in 2009 implies a poor detection of seasonal variability. We also did not ask for detailed information about the number of patients or the number of cases during the study periods – thus the true prevalence could not be calculated. Despite these limitations, the regional clustering of certain types observed in 2008 was confirmed in 2009. Some of the remaining questions will be answered by the voluntary reporting system, but since all laboratories are not yet participating, it is too early draw any conclusions from this system.

The proportion of *C. difficile* isolates with reduced susceptibility to moxifloxacin was 16% in Sweden 2009 and thus less than half of the 37.5% reported in Europe in 2005 [5]. Decreased susceptibility to moxifloxacin

Figure 6

Extent of prescription of moxifloxacin by county, Sweden, 2008 and 2009



has been found in several *C. difficile* types isolated in Europe and worldwide, and appears to be common in dominant types including 001, 018, 027 and 106 in, for example, England [7], Scotland [27], Germany [28] and Italy [5]. We found decreased susceptibility mainly in types 012, 017, 046 and 231/SE37. Type 012 is related to serogroup C [29] and has been reported as a common type in Sweden, Greece and Hungary [2,19] while toxin A-negative, toxin B-positive type 017 has been found to be associated with outbreaks in, for example, Poland, Canada and the Netherlands [6,30]. Regarding type 231/SE37, the proportion of this type was 0.8 % at the Karolinska University Hospital, Huddinge, Sweden, in 1999 [4], but there is no information on whether this type has caused any outbreaks in other countries.

Despite reports of increased prevalence of moxifloxacin resistance in *C. difficile* [5,31-33], including outbreaks of *C. difficile* 027 occurring shortly after replacing levofloxacin with moxifloxacin/gatifloxacin [34], it is currently unclear whether moxifloxacin use implies higher risk for *C. difficile* infection than other commonly used antibiotics [35-38]. Thus, we can neither confirm nor exclude the possibility that moxifloxacin has contributed to the selection of types 046 and 012 in certain Swedish counties. In this context, it should be pointed out that types 012, 017, 046 and 231/SE37 had decreased susceptibility also to clindamycin and erythromycin. Moreover, type 012 was shown to be resistant to many other antibiotics that were not tested in this report [32]. Thus, their success as pathogens may just reflect general selection due to the overall antibiotic use in healthcare settings [7]. Nevertheless, the cause for the variable prescription rates of moxifloxacin in Sweden needs to be investigated further.

In conclusion, in our two nationwide *C. difficile* surveillance studies in Sweden in 2008 and 2009, there was a geographical clustering of PCR-ribotypes 012, 017, 046 and a novel type (231/SE37), suggesting transmission and local outbreaks. Our results support the need for a central European or international *C. difficile* database with capability to continuously report and characterise emerging types, as well as a national surveillance system to monitor and control *C. difficile* incidence, type distribution, resistance pattern and related antibiotic use.

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New calls for proposals in the field of health launched by the Executive Agency for Health and Consumers

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On 3 March 2011 the Executive Agency for Health and Consumers (EAHC) launched the calls for proposals for joint actions, operating grants, projects and conferences based on the 2011 Work Plan of the Health Programme adopted on 22 February 2011.

These calls for proposals are open to all legally established organisations such as non-governmental organisations, public sector bodies, public administrations, universities, higher education establishments, etc. established in one of the 27 EU countries or in EFTA countries members of the European Economic Area (Iceland, Liechtenstein, and Norway) or in Croatia.

The deadline for submitting applications is **27 May 2011**.

For receiving a European grant, projects generally have to contribute at least to one of the three main objectives of the Health Programme (2008-2013): to improve citizens' health security, to promote health, including the reduction of health inequalities, to generate and disseminate health information and knowledge.

The 2011 work plan gives more emphasis and resources to a focused cooperation with the European Union (EU) Member States. The amount of EUR 17,040,000 will be dedicated to the funding of five joint actions: (i) health technology assessment, (ii) e-health, (iii) organ transplantation, (iv) patient safety, and (v) rare diseases.

Normally, for projects and grants, up to 60% of the eligible costs can be covered by the EU contribution and at least 40% of project costs must be funded from partners' sources. In cases of exceptional utility, the EU contribution can go up to 80% of the eligible costs. In case of joint actions, the EU contribution cannot exceed 50%, excepting cases of exceptional utility where it can go up to 70%. Exceptional utility may occur when activities have a very significant European added value in the areas indicated in the work plan. Conferences will be eligible for co-financing by the EU of up to EUR 100,000 with a maximum 50% of the total budget of the conference.

More information is available on the website of the Executive Agency for Health and Consumers at the following address: http://ec.europa.eu/eahc/health/index.html