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West Nile virus: the need to strengthen preparedness in Europe

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The ongoing outbreak of West Nile virus (WNV) infections in humans in Greece described in this issue of *Eurosurveillance* is a timely reminder that WNV is a re-emerging pathogen in Europe [1]. So far, WNV has been documented in animals and humans in several countries across Europe, mainly in central Europe and in the Mediterranean region. Over the last 15 years, outbreaks in horses and/or humans were reported from Romania, Hungary and Portugal, Spain, France, Italy and Greece [2].

In 2010, a single probable human case was reported in July in Portugal. Outside the European Union (EU), WNV circulation has been documented in horses in Morocco and human cases have occurred in Russia (Volgograd Oblast) and in Israel. All these regions are located along the main routes of migratory birds. The current outbreak in humans in northern Greece, is the first recognised WN fever outbreak in humans in this country. However, studies suggest that WNV has probably been circulating in humans in the region of central Macedonia in northern Greece for many years [3,4].

West Nile fever is a viral disease transmitted by mosquitoes and is distributed worldwide. The primary cycle of WNV involves ornithophilic mosquitoes and birds; some mosquito species mostly from the Culex genus can bite infectious birds and subsequently transmit the virus to humans and/or horses during another blood meal. Humans and horses are considered as dead-end hosts. The vast majority of human cases remain asymptomatic after infection and severe neuroinvasive illness is reported in less than 1% of the patients. The main risk factor for severe clinical presentation is to be an elderly person. In this age group, reported case fatality rates may reach 10% [5]. In addition the high number of non-symptomatic cases may increase the risk of WN virus transmission through blood donation or organ transplants [6].

Each WNV outbreak is unique in that there is a complex interaction of different factors in space and time that contribute to the transmission of the virus to humans. These factors range from the introduction of infected migratory birds into native local bird populations, to climatic factors that increase the abundance of competent mosquito vectors and bridge vectors, to changes in human behaviour that favour exposure to infected mosquitoes. It is this complexity that makes each WNV outbreak particular and that make development and implementation of preparedness plans for the prevention of cases in humans so difficult.

The recently reported probable and confirmed cases of WNV infection in Portugal and Greece, respectively reconfirm that this virus is actively circulating in several countries in the EU and that transmission to humans can be expected on a regular basis during the mosquito season. Also, reports of sporadic cases from several regions in Hungary during previous years indicate that WNV activity is widely distributed throughout this country and not limited to a single focus [7]. A recent study in Italy linked to infected organ donors [8] draws the same conclusion, that the virus is being transmitted in areas previously thought to not be at risk or affected. Furthermore, the case report in this issue of a Dutch traveller returning from Israel with WN infection highlights the need for awareness among physicians and laboratory staff to consider WNV infections as a differential diagnosis in cases where patients return from areas where they may have been exposed to the virus [9].

The events described above strengthen the need for integrated multidisciplinary surveillance systems and response plans. This includes raising the awareness of clinicians and veterinarians of the clinical presentation of WNV disease in humans and horses (particularly during the mosquito season from June to October), primarily in areas considered as at major risk surrounding irrigated areas and river deltas. Furthermore, strengthening the understanding of suitable habitats for birds that would increase the bird-mosquito-human interface would be of value. In terms of entomology, a thorough understanding of competent vector species, their breeding ecology, their abundance and geographic range is of significant importance in establishing limits around WNV affected areas and in the identification of potential new at-risk areas.

In addition, there is a need to have a better and more precise picture of WNV risk areas in Europe and neighbouring countries in order to implement appropriate control measures, especially guidelines for blood donation and organ transplants. Also, studies in Europe are required to better understand the cycle of transmission and the maintenance of WNV in the environment over the years to provide appropriate indicators for risk assessment.

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Ongoing outbreak of West Nile virus infections in humans in Greece, July – August 2010

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Article published on 26 August 2010

Between early July and 22 August 2010, 81 cases of West Nile neuroinvasive disease were reported in the region of Central Macedonia, northern Greece. The median age of cases was 70 years. Encephalitis, meningoencephalitis or aseptic meningitis occurred mainly in patients aged 50 years or older. This is the first time that West Nile virus (WNV) infection has been documented in humans in Greece. Enhanced surveillance and mosquito control measures have been implemented.

Introduction

On 4 August 2010, physicians from the Infectious Disease Hospital in Thessaloniki, northern Greece, informed the Hellenic Centre for Disease Control and Prevention (KEELPNO) about an increase in the number of hospitalised cases with encephalitis in the previous month (13 patients with encephalitis were hospitalised in July 2010, compared with a mean of five hospitalised cases in the same month of the previous three years). Despite several laboratory tests, no aetiological factor had been identified. Most patients were elderly (over 65 years of age) and resided in the region of Central Macedonia, northern Greece. On the same day, 11 serum and three cerebrospinal fluid (CSF) specimens from 11 patients with encephalitis and/or aseptic meningitis were sent for further testing to the Reference Laboratory for Arboviruses at the Aristotle University of Thessaloniki. The following day, the results showed that IgM antibodies against West Nile virus (WNV) had been detected in 10 of the 11 serum specimens and in all three CSF specimens. WNV infection in humans had not been previously documented in Greece.

WNV is a positive-sense RNA virus of the *Flaviviridae* family, belonging to the Japanese encephalitis antigen group of viruses [1]. WNV is maintained in an enzootic cycle between birds and mosquitoes, mainly *Culex* species, while humans, horses and other mammals are incidental or dead-end hosts. Most human WNV infections are subclinical, and approximately 20% of infected individuals develop a febrile illness, while in less than 1%, the disease progresses to neuroinvasive disease, with the most severe form seen among elderly and immunocompromised individuals [2].

Although the virus was first isolated in 1937, interest in its impact on humans increased in 1996, when a large outbreak of West Nile neuroinvasive disease (WNND) was observed in Romania and in 1999, when WNV was introduced into the United States [3,4]. Several cases of WNV infection have been reported in horses and humans in Mediterranean countries [5-8], while WNND has been recently reported in humans in Hungary and Italy [7-9].

Methods

Surveillance

Following an alert issued by the Ministry of Health and KEELPNO on 6 August 2010 about 11 reported WNV infection cases, physicians in Greece were asked to notify KEELPNO of all confirmed or probable cases of WNV infection using a standardised reporting form, which included information on the demographic characteristics, clinical manifestations, underlying chronic medical conditions, potential risk factors and laboratory results of cases. The exact address of cases' place of residence was obtained from hospital registries. In addition, active surveillance to identify cases included daily telephone inquiries to the hospitals of the region of Central Macedonia, from where the cases had been reported.

Case definition

The 2008 European Union case definition of WNV infection [10] was used, with slight modifications. A confirmed case was defined as a person meeting any of the following clinical criteria: encephalitis, meningitis, fever without specific diagnosis and at least one of the four laboratory criteria: (i) isolation of WNV from blood or CSF, (ii) detection of WNV nucleic acid in blood or CSF, (iii) WNV-specific antibody response (IgM) in CSF,

and (iv) WNV IgM high titre, and detection of WNV IgG, and confirmation by neutralisation.

A case was considered probable if the patient met the above clinical criteria and a WNV-specific antibody response was demonstrated in his or her serum sample. Epidemiological criteria were not used in the case definition due to the absence of recent surveillance data in animals.

Laboratory methods

Serum and CSF specimens were tested for the presence of WNV-specific IgM and IgG antibodies using commercial ELISA kits (Focus Technologies, Cypress, CA, USA). Reverse transcription-polymerase chain reaction (RT-PCR) was performed on RNA from 15 of 99 specimens, because the remaining samples had been taken between three and 15 days after the onset of illness, when viraemia is usually over. Primer sets specific for WNV and degenerate primers (able to detect flavivirus RNA) were used [11,12].

Data analysis

Data were entered in a database designed using Epidata software (Epidata association, Denmark, version 3.1) and were analysed using the GNU R software environment. Incidence was calculated using the 2007 mid-year estimated population from the Hellenic Statistical Authority as denominator [13].

Results

By 22 August 2010, 99 cases of WNV infection had been notified to KEELPNO. Of these, 81 had central nervous system manifestations (West Nile neuroinvasive disease, WNND) and 18 (eight probable and 10 confirmed cases) had only mild symptoms of fever and headache. We analyse here the 81 cases of WNND. Of these, 39 were confirmed and 42 were probable cases. The overall incidence of WNND was 0.72 cases per 100,000 population.

In total, 77 serum and 47 CSF specimens were available; for 45 of the 81 WNND patients both CSF and serum specimens were provided, while for four patients only CSF was available. WNV-specific IgM antibodies were detected in all 77 serum and in 39 of the 47 CSF specimens, while WNV-specific IgG antibodies were detected

Reported cases of West Nile neuroinvasive disease by date of symptom onset, Greece, 1 July – 22 August 2010 (n=81)
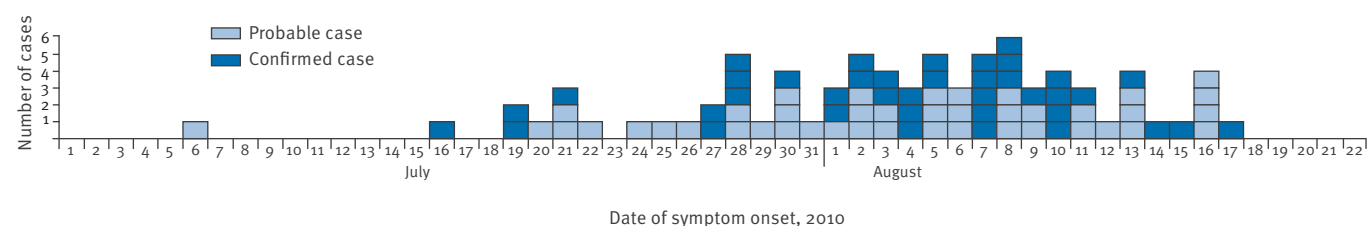

Date of symptom onset, 2010

Characteristics of reported cases of West Nile neuroinvasive disease, Greece, 1 July – 22 August 2010 (n=81)

| Characteristic	Number of cases	Incidence (per 100,000 population)	Risk ratio (95% CI)
Age group (years)			
<20	3	0.14	Reference
20–29	3	0.20	1.44 (0.96–10.95)
30–39	2	0.11	0.83 (0.18–7.37)
40–49	2	0.12	0.90 (0.29–9.85)
50–59	12	0.85	6.16 (1.93–29.40)
60–69	14	1.18	8.56 (1.29–33.72)
70–79	37	3.49	25.39 (6.92–101.72)
≥80	8	1.79	13.05 (2.81–43.94)
Sex			
Female	36	0.64	Reference
Male	45	0.81	1.27 (0.92–3.24)
District (prefecture) of residence			
Thessaloniki	27	2.37	Reference
Imathia	21	14.57	6.15 (1.32–11.64)
Kilkis	9	10.44	4.44 (1.29–12.34)
Pella	17	11.72	4.95 (2.12–8.14)
Pieria	2	1.56	0.66 (0.32–5.43)
Serres	3	1.59	0.67 (0.17–3.25)
Larisa	2	0.70	0.30 (0.12–1.85)
Total (in country)	**81**	**0.72**	–

CI: confidence interval.

in 42 of the 77 serum and 17 of the 47 CSF specimens. In 39 of the 45 patients for whom both types of specimen were available, the presence of IgM in both CSF and serum was seen, proving autochthonous antibody production; for IgG this was not tested. As cross-reactions are common among flaviviruses, specimens were also tested for tick-borne encephalitis (TBE) virus (although TBE is not prevalent in the area and none of the patients reported tick bites): all were negative. Low cross-reactivity was seen with dengue virus; however, when a positive result was obtained for dengue virus, the titres were very low compared with the high titres seen for WNV. None of the patients had been vaccinated for yellow fever. RT-nested PCR was negative in all specimens tested.

The first cases of WNND had onset of symptoms in early July (Figure 1).

FIGURE 2

Place of residence of reported cases of West Nile neuroinvasive disease, Greece, 1 July – 22 August 2010 (n=80)[a]
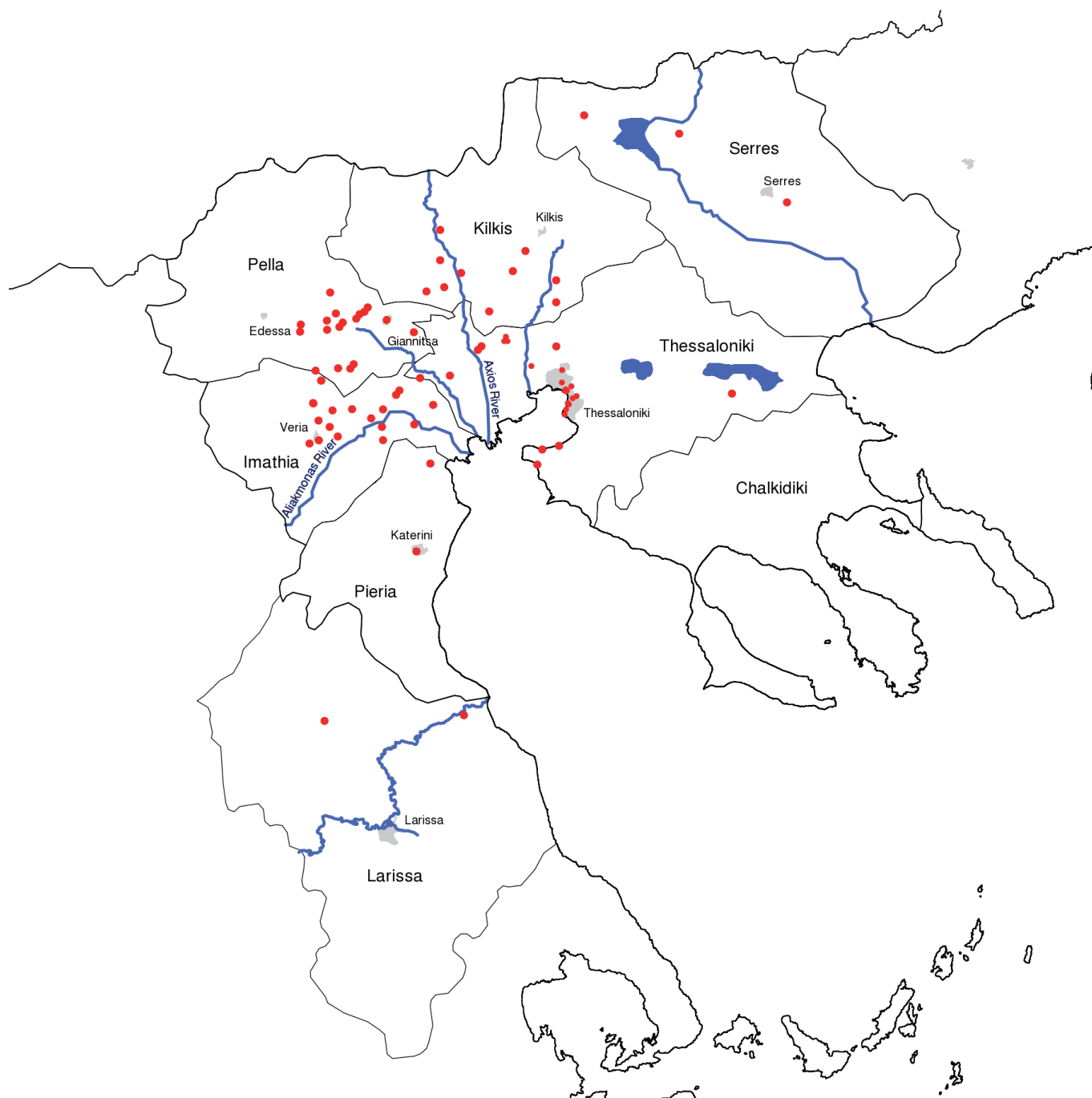
[a] For one of the 81 cases of West Nile neuroinvasive disease, the place of residence has not been confirmed and is not included on the map. Each dot represents one case; the grey areas represent towns or cities; the blue lines represent rivers.
The district of Larissa belongs to the region of Thessalia. All other districts from where cases have been reported belong to the region of Central Macedonia.

The median age of the WNND cases was 70 years (range: 12–86 years), with most (n=71) aged 50 years or older (Table 1). Of all WNND cases, 45 (56%) were males.

The incidence, 1.7 per 100,000 population, of WNND among those aged 50 years or older was almost 12 times higher (risk ratio: 12.2; 95% confidence interval: 4.9 to 28.5) than that of individuals aged under 50 years. The risk of WNND was 27% higher among males compared with females (Table 1).

The place of residence of the WNND cases is presented in Figure 2: 30 resided in an urban area and 51 in a rural setting. The vast majority (n=79) lived in districts (prefectures) of the region of Central Macedonia and only two were reported from the region of Thessalia (district of Larissa). It is of note that a large number of cases (n=58) lived near rivers and/or on the irrigated plains between and surrounding the Aliakmonas and Axios rivers (Figure 2).

None of the notified cases reported travel to a known WNV-endemic area during the two weeks before onset of symptoms. Information on outdoor activities was gathered from 55 of the cases: 27 cases reported spending many hours outdoors in the countryside every day. None of the cases had a history of blood transfusion or tissue/organ transplant during the two weeks before the onset of symptoms.

All notified cases with central nervous system manifestations were hospitalised. Of those, 65 had encephalitis or meningoencephalitis, and 16 had aseptic meningitis (Table 2).

Of the 65 WNND cases with encephalitis and/or meningoencephalitis, 60 were aged 50 years or older.

Table 2

Clinical manifestations of reported cases of West Nile neuroinvasive disease by age group, Greece, 1 July – 22 August 2010 (n=81)

Age group (years)	Number of cases with encephalitis or meningoencephalitis	Number of cases with aseptic meningitis
<20	1	2
20–29	2	1
30–39	1	1
40–49	1	1
50–59	9	3
60–69	13	1
70–79	31	6
≥80	7	1
Total	65	16

Information on underlying chronic medical conditions was available for 60 of the 81 patients. These included hypertension (n=26), a history of immunosuppresion (n=17), coronary artery disease (n=11), and diabetes mellitus (n=9). Ten cases were admitted to an intensive care unit. As of 22 August 2010, eight cases had died, giving a case fatality rate of 9.9% among the reported WNND cases. All deceased patients were aged over 70 years, and suffered from hypertension and diabetes.

Discussion and conclusions

We describe here 81 cases of WNND reported between early July and 22 August in the region of Central Macedonia, northern Greece. The fact that a small proportion of infected individuals develop WNND [2] suggests that this ongoing outbreak may in fact be larger. As of 26 August 2010, more cases of WNND have been reported bringing the total number of WNND cases to 108. In addition, information was gathered through enhanced surveillance, and a degree of under-reporting, particularly at the beginning of the outbreak, is expected.

Serological surveys conducted in humans in the 1980s and in 2007 in Greece identified WNV antibodies in approximately 1% of selected populations (i.e. farmers, wood-cutters, shepherds) in the region of Central Macedonia. Of 392 serum samples collected from residents in a selected urban area in the district of Imathia (central Greece) in 2007, six were positive for WNV, of which four were confirmed by microneutralisation assay [14,15]. The authors concluded that WNV or related viruses circulate in endemic cycles in rural areas in Greece. In contrast, a survey of 9,590 blood donations and 115 CSF samples from patients with aseptic meningitis in Greece between 2005 and 2007 revealed no positive results for WNV by nucleic acid test. However, the sources of the clinical samples were major laboratories and/or blood banks in the cities of Athens and Ioannina [16].

The presence of WNV in animals is not monitored routinely in Greece. However, a few *ad-hoc* studies have been conducted. In a seroprevalence survey in animals in 1980, antibodies to WNV were found in 8.8% of sheep, 8.7% of goats, 3.9% of cattle, 20.4% of horses, 1.4% of pigs, and 24.5% of birds [17]. In an unpublished survey conducted from May 2001 to December 2004, 302 of 7,549 (4%) equine serum samples were found positive for WNV using neutralisation tests; positive samples in equines were found in 36 of 49 districts (prefectures) studied from all parts of Greece (O. Mangana, Ministry of Agriculture, personal communication, 10 August 2010).

In conclusion, previous studies in humans and animals suggest that WNV has probably been circulating in the region of Central Macedonia and possibly in other parts of Greece for many years. Increased rainfall, high temperatures and humidity during recent months, as well as the geographical features (i.e. river deltas, rice

fields, irrigated plains) of some parts of the region of Central Macedonia, have probably favoured the multiplication of *Culex* species, leading to the occurrence of numerous cases of WNV infection in humans.

Public health measures

After the initial notification of the cluster of WNV infection cases, the Hellenic Centre for Disease Control and Prevention alerted physicians throughout the country and prepared a set of guidelines for health professionals, with the necessary instructions for laboratory diagnosis. Surveillance of human WNV infection cases has been established and the public has been given guidance about personal protective measures.

The Hellenic Centre for Blood Transfusion prepared specific guidelines and measures for blood and blood products safety, including a 28-day deferral policy for all donors residing in the specific districts (prefectures) where WNV infection cases were detected, as well as any blood donor who had visited the same districts (prefectures) for one or more days. Blood units that had been collected in the same region since the beginning of July were quarantined until tested for WNV by PCR. They were all found negative for WNV and were released for use. Finally, specific advice was provided to all blood donors asking them to inform the blood donation department they attended, if they develop a fever within 15 days after their donation. The National Organisation for Transplantations has also been informed and is requesting WNV testing of donors depending on residence area or travel history.

The Ministry of Health and Social Solidarity coordinates the intensification of mosquito control programmes at district level, which are already being implemented. The Ministry also undertook the coordination of the health sector preparedness and the collaboration with the veterinary services of the Ministry of Agriculture. Surveillance for WNV in mosquitoes (using bait traps) has been put in place.

Updates on reported WNV infection cases and deaths in Greece are published in the daily epidemiological surveillance reports (available in English from http://www.keelpno.gr/eng/wnv).

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Retrospective screening of solid organ donors in Italy, 2009, reveals unpredicted circulation of West Nile virus

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Since the occurrence of West Nile virus (WNV) infection in humans in 2008 in Italy, concerns have been raised about the potential risks associated with solid organ transplantation (SOT). A nationwide retrospective survey showed that 1.2% of SOT donors in 2009 were WNV-seropositive and demonstrated that human WNV infection is distributed throughout several Italian regions. Transmission of WNV or other arboviruses through SOT is a possibility and risk assessment should be carried out before SOT to avoid infection through transplantation.

Background

In 1998, when the first cases of equine West Nile virus (WNV) infection in Italy were detected in Tuscany, no human cases were reported [1]. WNV re-emerged in Italy in 2008, and viral circulation was identified among different vectors and different animal species, including horses and wild birds [2]. The first cases of human WNV neuroinvasive infections in Italy were identified in September 2008 in the Emilia-Romagna region [3].

In the summer of 2009, additional human cases were reported in the same and in other neighbouring Italian regions. In one of the most affected areas in the province of Ferrara, a WNV seroprevalence of 0.68% was observed in healthy blood donors, raising concerns about the potential risks of WNV transmission by blood transfusion and organ transplantation [4,5].

In 2009, as a precautionary measure, the National Transplant Centre issued guidance that potential donations from all donors of solid organs, tissues and cells from the Bologna, Ferrara, Modena and Reggio-Emilia provinces who were screened by nucleic acid amplification tests (NAAT) for the presence of WNV viraemia, and tested positive, had to be rejected. Donors from other regions who had spent at least one overnight stay in the above-listed provinces during the 28 days before donation should not be considered eligible for donation [6].

Two days before screening of donors was implemented, in Bologna, Emilia-Romagna province, transmission of WNV infection through liver transplantation was detected by NAAT before clinical symptoms appeared in the recipient. The transmission of WNV was managed, post-transplant, by administration of hyperimmune serum and viraemia-guided adjustment of the immunosuppressive drug regimen, accompanied by supportive care [7]. The patient recovered from the transplantation and did not develop symptoms of WNV infection or sequelae. Before this case, all reported SOT donor-derived WNV infections were identified retrospectively in symptomatic transplanted patients with severe outcomes in the United States (US) [8]. This post-transplant detection of WNV infection in SOT recipients demonstrates that the absence of a universal screening policy in the immediate pre-transplant period makes it almost impossible to accurately quantify the transmission rate and the subsequent clinical impact of WNV transmission to SOT recipients. In the US, a recent serosurvey carried out among SOT recipients suggested that asymptomatic WNV infection may be common in areas of WNV activity and that the severe clinical presentations of WNV infections are equally frequent in both immunocompromised and immunocompetent subjects [9].

On the basis of this evidence, re-defining the risks for WNV transmission by organ, tissue and cell transplantation was identified by the National Transplant Centre

as a necessary safety measure before the start of the next WNV season, from late spring to early autumn. The Italian Transplant Network considered this to be a priority in order to establish the factual basis for implementing future strategies for preventing WNV transmission.

A nationwide retrospective survey of WNV seroprevalence was therefore undertaken in all SOT donors recruited by the National Transplant Centre during 2009.

Methods

Serum samples from the Italian SOT donors in 2009 stored in the biorepository facilities of the Italian Transplant Network, were analysed by the two reference laboratories identified by the National Transplant Centre for assessment of WNV infection: the Laboratory of Virology at the National Institute for Infectious Diseases 'L. Spallanzani' in Rome and the Regional Reference Centre for Microbiological Emergencies of the Microbiology Unit, St Orsola–Malpighi University Hospital, in Bologna.

The presence of WNV-specific IgG and IgM was investigated using a two-step approach: first, a screening test was performed using a commercial enzyme-linked immunosorbent assay (ELISA) method (Euroimmun, AG, Lübeck, Germany); all samples that were positive for either IgG or IgM were then confirmed by an immunofluorescent antibody assay (IFA, Euroimmun). Second, the IgG-positive sera were further evaluated by ELISA to measure the IgG avidity, using the method of Fox *et al.* [10]. All the IgG- and IgM-positive samples identified in the previous steps were further characterised by microneutralisation assay (MNTA) against WNV as previously described [11], and to rule out possible cross-reactions of the test, serum samples were also tested by MNTA against Usutu virus. All IgM-positive sera samples were retrospectively tested by NAAT using Procleix-WNV assay performed on the TIGRIS system, Novartis (for donors from Emilia Romagna) or Artus Real Art *WNV LC* RT RCRt kit, QIAGEN (for donors from Piedmont and Tuscany) in order to evaluate the presence of WNV viraemia. During the screening activity for WNV in 2009 no WNV-positive donor was identified before SOT.

Results

A total of 1,248 serum samples from SOT donors in 2009 were analysed, accounting for 98.1% of SOT donors recruited during that year. Table 1 lists the number of SOT donors evaluated in this study, by region of residence. WNV-specific antibodies were detected in 15 samples from individual donors at the time of organ donation, thus giving an overall positivity rate of 1.2%. Data from MNTA indicate that seven of 15 samples had

TABLE 1

Region of residence of solid organ donors tested for West Nile virus, Italy, 2009 (n=15)

Region	Number of donors tested	Number of donors positive WNV[a]
Abruzzo-Molise	12	0
Basilicata	11	1
Calabria	12	0
Campania	74	0
Emilia Romagna	120	4
Friuli-Venezia Giulia	45	1
Lazio	103	1
Liguria	33	0
Lombardy	226	0
Marche	51	0
Piedmont-Aosta Valley	120	2
Bolzano-Bozen independent provincial administration	10	0
Trento independent provincial administration	17	0
Apulia	44	0
Sardinia	24	0
Sicily	59	0
Tuscany	167	6
Umbria	11	0
Veneto	109	0
Total	**1,248**	**15**

WNV: West Nile virus

[a] IgG - and/or IgM-positive, by serological screening (see Table 2).

neutralisation activity against WNV. However, eight samples did not show appreciable neutralisation titre against WNV. Among these, two samples were reverse transcription-polymerase chain reaction (RT-PCR) positive and two other samples probably showed neutralisation activity against the related Usutu virus (Table 2).

The inability to confirm by MNTA all the samples testing positive by ELISA and IFA might be due to the cross-reactivity to closely related flaviviruses or might be consistent with the notion that the neutralising response to WNV in humans is variable and that only a subset of infected individuals generate antibodies against high-neutralising epitopes [12]. Furthermore, from a technical point of view, maturation state of WNV particles used in the MNTA might have been important for determining whether antibodies in a given serum samples were judged WNV-specific by MNTA, as already reported by Nelson *et al*. [13].
The highest number of WNV-seropositive donors were from the regions of Emilia-Romagna and Tuscany (Table 1 and Figure).

Table 2 shows the place of residence, demographic information and laboratory results obtained for these 15 patients.

Two donors (from Piedmont and Emilia-Romagna) were identified as IgG- and IgM-positive. One donor, from Tuscany, was positive only for IgM. All these three IgM-positive samples were also positive for viral RNA, showing quite a low viral load (equivalent to a genome copy number of ≤3 log copies/ml). We speculate that such a low level of viral concentration in blood is likely to pose a limited risk of viral transmission through SOT. Four IgG-positive specimens showed an antibody avidity of 80% to 90% suggesting that exposure to WNV probably occurred more than six months before the organ donation; another four IgG-positive samples (all from donors who donated organs after June 2009) showed an antibody avidity lower than 40 %, suggesting that the infection was probably acquired within six months before the organ donation, a time frame consistent with WNV exposure during the 2009 mosquito season (June to October).

In addition, recipients of a solid organ from donors who were shown to have been positive for WNV genome or to have had IgM-specific antibodies at the time of organ donation were also included in this study. Two recipients from the Piedmont IgM-positive donor (one kidney recipient and one liver recipient) did not show any seroconversion to WNV. The remaining recipient (who received a kidney) was not available for testing. All three recipients are well and have never shown signs consistent with WNV infection. The IgM-positive donor from Tuscany did not in the end donate organs for reasons not related to WNV infection.

Discussion and conclusions

The occurrence of an antibody response in some donors from the Piedmont, Friuli-Venezia Giulia, Marche and Basilicata regions is rather unexpected and shows evidence of WNV infection in humans in several Italian regions. However, it is possible that WNV activity has hitherto been underestimated in some regions, due to an insufficient veterinarian and entomological surveillance system, which may have generated inconsistent data. Evidence from the present study – i.e. the low IgG avidity index, the presence of an IgM-specific response and/or WNV RNA in blood samples – is consistent with the notion that recrudescence of WNV activity in Italy occurred in the last two years, following the report of the first human cases of neuroinvasive disease [3-5]. These findings highlights the need for an accurate nationwide approach to risk assessment related to transplantation, in order to implement appropriate prevention strategies and limit the potential burden of severe neurological complications in the immunocompromised recipients. During the 2009 season, only one case of WNV transmission by SOT was observed in the Emilia-Romagna region [7]. No additional cases of WNV transmission from infected donors were documented retrospectively in our study, suggesting that the transmission of WNV to recipients of SOT from viraemic donors does not always occur. Furthermore,

the positivity in MNTA against Usutu virus in two samples additionally confirms that this virus has to be added to the list of those that can be transmitted to humans [14,15] and its possible transmission through SOT deserves particular attention.

These results indicate that, due to the well-known circulation of WNV in many different areas in Italy, transmission of WNV or other arboviruses through SOT is possible, and that the risk assessment process related to transplantation is a challenging issue that requires a systematic approach [7].

Regional Coordinators of the Italian Transplant Network:
M Scalamogna (Milan); A Famulari (L'Aquila); A Saracino (Matera); B Giacon (Bolzano); P Mancini (Reggio Calabria); E Di Florio (Naples); L Ridolfi (Bologna); R Peressutti (Udine); D Adorno (Roma); A Gianelli Castiglione (Genoa); S Vesconi (Milan); D Testasecca (Ancona); A Amoroso (Turin); F Paolo Schena (Bari); C Carcassi (Cagliari); V Sparacino (Palermo); R Lippi (Florence); E Gabardi (Trento); C Gambelunghe (Perugia); F Calabrò (Padua).

TABLE 2

Detailed information of solid organ donors who tested positive by serological screening for West Nile virus

Region	Sampling date, 2009	Cause of death	Age at death (years)	Place of residence (province)	Possible place of exposure[a] (if different from place of residence)	WNV- specific antibody titres		IgG avidity (%)[b]	MNTA titre against WNV[d]
						IgG	IgM		
Basilicata	22 Sep	Stroke	64	Matera	Lake Garda	≥1:160	<1:10	89	1:40
Lazio	7 Jul	Stroke	67	Rome	–	1:80	<1:10	31	1:40
Tuscany	3 Jan	Cranial trauma	57	Lucca	–	1:80	<1:10	NA	<1:10
Tuscany	26 Mar	Brain haemorrhage	67	Versilia (Lucca)	–	1:40	<1:10	NA	<1:10
Tuscany	15 Jul	Stroke	87	Florence	–	<1:10	1:10[c]	NA	<1:10
Tuscany	25 Aug	Brain haemorrhage	74	Siena	–	1:80	<1:10	11	1:20
Tuscany	20 Oct	Brain haemorrhage	40	Prato	–	≥1:160	<1:10	43	<1:10
Tuscany	13 Nov	Cranial trauma	85	Livorno	–	≥1:160	<1:10	68	1:80
Piedmont	28 Jul	Asphyxia/hypoxia	57	Mottalciata (Biella)	–	1:80	<1:10	73	<1:10[e]
Piedmont	21 Oct	Post-anoxic coma	63	Chieri (Turin)	–	1:160	1:40[c]	13	<1:10
Emilia Romagna	23 Feb	Heart failure	71	Modena	–	1:400	<1:10	88	1:80
Emilia Romagna	14 May	Brain haemorrhage	56	Traversetolo (Parma)	–	1:200	<1:10	52	<1:10[f]
Emilia Romagna	1 Sep	Brain haemorrhage	78	Naples	Reggio Emilia	>1:1600	1:80[c]	25	1:160
Marche	1 Aug	Brain haemorrhage	48	Rimini	–	1:400	<1:10	84	1:20
Friuli-Venezia Giulia	24 Jul	Cranial gunshot wound	63	Gorizia	–	1:1600	<1:10	88	<1:10

MNTA: microneutralisation assay; NA: not applicable, due to low or negative IgG enzyme-linked immunosorbent assay (ELISA) optical density values; WNV: West Nile virus.

[a] For the donors whose place of residence was not in the WNV activity area identified in 2008 and 2009, the possible place of WNV exposure was provisionally assigned by a retrospective investigation based on consultation of medical records or on direct information received by the donors' relatives. The dashes (–) in this column indicate that no history of travel in WNV-endemic areas other than those identified in Italy was recorded in the 12 months before donation.

[b] <40% : less than 6 months after infection; >60% : 6 months or more after infection, according to [10].

[c] Positive for WNV by reverse transcription-polymerase chain reaction (RT-PCR).

[d] Samples yielding a neutralisation titre ≥ 1:20 were scored as positive.

[e] MNTA titre against Usutu virus 1:40.

[f] MNTA titre against Usutu virus 1:80.

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Case report: West-Nile virus infection in two Dutch travellers returning from Israel

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We report about West Nile virus (WNV) infections in a symptomatic traveller returning from Israel and in her asymptomatic travel companion. Knowledge of the current epidemiological situation in Israel from where WNV cases were reported recently enabled a rapid diagnosis. The described cases serve as a reminder for physicians to consider WNV in the diagnosis of patients returning from areas with potential circulation of the virus.

At the end of July 2010, a Dutch woman in her early thirties presented to our first aid department with fever, retro-orbital headache and a macular rash. The day before she had returned from a 10-day holiday to Israel where she noticed several mosquito bites during a camping trip at the Sea of Galilee. There was no history of tick bites. Five days before presentation, she had suddenly fallen ill with extreme fatigue, myalgia, fever and an increasingly severe headache. Movement of the eyes had been painful. The next day she had developed a burning sensation of the skin that was followed by a skin eruption. The rash started on the trunk and spread to arms and legs.

Upon presentation in the first aid department she did not appear ill. Body temperature was 36.4°C. There was a generalised macular rash, sparing hands and feet, and no petechiae or eschars were present. Neurological examination revealed no abnormalities. Potential infection with West-Nile virus was suspected because of the clinical picture and recent reports of West Nile virus cases in Israel [1].

Laboratory examination showed a leukopenia of 1.8×10^9/L with a predominance of large granular lymphocytes; thrombocyte count was 92×10^9/L. Infection with Epstein Barr virus, cytomegalovirus, dengue virus, enterovirus and parechovirus was excluded by serology and antigen test or PCR. A lumbar puncture was not performed due to missing neurological symptoms. West Nile virus RNA could not be detected in the EDTA plasma sample taken on day five after onset of disease by a TaqMan reverse transcriptase-PCR assay, using a probe for the WN3'NC [2]. However, in a second blood sample obtained fifteen days later, seroconversion for both serum IgG and IgM antibodies against

West Nile virus was detected with an indirect qualitative enzyme-linked immunosorbent assay (ELISA) (Focus Diagnostics, Cypress, California). This assay uses antibody capture technique for the detection of IgM antibodies.

Once the diagnosis was confirmed, serology was performed in the patient's travel companion who reported having had similar complaints, but who had already recovered by the time he returned to the Netherlands. The laboratory results showed that he was seropositive for WNV IgG and IgM. Recovery was uneventful in both patients.

West-Nile virus is endemic in Israel. The largest recent outbreak in humans in Israel occurred in the year 2000 with more than 400 reported cases [3]. Recently, 12 cases of West Nile fever have been reported, centered around the Tel Aviv area [1]. C*ulex perexiguus, Cx. pipiens*, and *Aedes caspius* are the vectors of West Nile virus in Israel where most cases of West Nile fever occur between August and October. The seasonal occurrence of human cases reaches a peak one month after the mosquito peak [4].

Measures to avoid mosquito bites such as wearing protective clothes and using repellents are recommended during the whole transmission season and this case report serves as a reminder to physicians to consider West Nile fever in patients with fever returning from Israel.

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Outbreak of invasive meningococcal disease in Goleniów County, north-west Poland, March 2009

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In March 2009, six cases of invasive meningococcal disease (IMD) were reported in two communes of Goleniów County in north-west Poland. The people affected were aged from seven to 25 years. The overall incidence rate in the county was eight per 100,000 population. All the patients recovered fully. No epidemiological links were established between the cases. All were infected with *Neisseria meningitidis* serogroup C, sequence type (ST) 11 and analysis of isolate DNA restriction fragment length polymorphism showed indistinguishable pulsed-field gel electrophoresis (PFGE) patterns. Sequencing of *porA* and *fetA* genes revealed that all isolates had PorA variant P1.5,2 and FetA variant F3-3. Based on epidemiological and microbiological data, the Polish Working Party on Meningococcal Infections took the decision to compulsorily vaccinate populations at highest risk in the region – primarily people aged from 6 to 19 years.

Introduction

Neisseria meningitidis, which in most instances asymptomatically colonises the human nasopharynx, may also cause rapid-onset septicaemia or meningitis – conditions that are referred to as invasive meningococcal disease (IMD). Although cases of IMD usually appear only sporadically, they may also emerge in clusters, outbreaks and large epidemics [1]. In Poland, IMD is a notifiable disease. Every suspected case has to be reported by physicians to the local Sanitary Inspectorate within 24 hours of hospital admission. All local Sanitary Inspectorates are then required to report the cases to the National Institute of Public Health - National Institute of Hygiene, which collates the information from the whole country. In addition to this mandatory notification, there is a laboratory-based surveillance system run by the National Reference Centre for Bacterial Meningitis. This centre collects all bacterial isolates from hospital laboratories as well as clinical materials (to be used if bacterial culture fails) from people with community-acquired invasive bacterial infections, including IMD. This activity is crucial for monitoring national trends in IMD and for detecting clusters or outbreaks of the disease, and thus enabling correct medical and epidemiological management.

Until 2009, *N. meningitidis* had been the most common laboratory-confirmed aetiological agent of community-acquired invasive bacterial infections in Poland (unpublished data). The incidence rate of IMD in the country is around one per 100,000 population [2-4]. As in some other countries (for example, Austria, Bulgaria, Denmark, Finland, Latvia and Norway), there is no mass vaccination against serogroup C meningococci in Poland [5].

This report describes an outbreak of IMD (n=6) in Goleniów County in the West Pomeranian region of Poland, close to the Polish–German border, in March 2009. It affected two communes, Goleniów and Przybiernów, with 33,000 and 5,180 habitants, respectively. The international Szczecin-Goleniów Airport is close to Goleniów, the capital of Goleniów County. West Pomerania has been severely affected by IMD in the past: from April 2003 to March 2004 the reported case-fatality rate of IMD caused by various meningococcal strains (of serogroups B, C and W-135) was very high: 43% (nine of 21 cases) [6]. More than half of the patients (n=13) developed meningococcal septicaemia, of whom nine died. Such a high number of cases with septicaemia accelerated the decision of Polish authorities to change the notification system. Since 2004, it has been obligatory to report all IMD cases, not only those with meningitis. Since then, the number of reported IMD cases in Poland started to increase. Until 2006, there were mostly sporadic cases, though there were some family clusters. However, in 2006 and 2007, at least five outbreaks of IMD, with high case-fatality rates, were notified in various parts of the country [7,8]. At the beginning of 2008, there was one outbreak in the Świętokrzyskie region (in the southeast) and then until the Goleniów County outbreak in March 2009, only sporadic cases had been reported in the country (unpublished data).

Methods
Case definition
In Poland, the definition of confirmed IMD case is similar to the European Union case definition of confirmed meningococcal case [9], encompassing patients meeting the following inclusion criteria:

- having symptoms of invasive bacterial disease (at least one of the following: fever, meningeal signs, petechial rash, septic shock or septic arthritis); and
- *N. meningitidis* isolated from a normally sterile site; or
- meningococcal DNA identified by polymerase chain reaction (PCR) in material from a normally sterile site; or
- Gram-negative diplococci detected in cerebrospinal fluid.

The Polish definition of confirmed IMD case does not include detection of *N. meningitidis* antigen in cerebrospinal fluid.

N. meningitidis isolation and characterisation

N. meningitidis was isolated from the six IMD cases in local hospital laboratories and identified according to their standard procedures. For subsequent characterisation, isolates were sent to the National Reference Centre for Bacterial Meningitis in Warsaw. Serogroups were determined by slide agglutination tests using commercial antisera (Remel). Serotypes and subtypes were evaluated by whole-cell enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies (from the National Institute for Biological Standards and Control, United Kingdom) [10]. Minimal inhibitory concentrations were assessed by the Etest method (bioMerieux).

The relatedness of isolates was determined by restriction fragment length polymorphism (RFLP) analysis of genomic DNA in pulsed-field gel electrophoresis (PFGE), using *Spe*I restriction enzyme for DNA digestion. The meningococci were also characterised by multilocus sequence typing and DNA sequencing of the *porA* and *fetA* genes, which encode outer membrane proteins [11,12]. Sequence types (ST) and PorA and FetA types were determined through the meningococcal typing website [13].

Results

Description of the outbreak

From 10 to 30 March 2009, there were six cases of IMD in Goleniów County. In Goleniów commune, there were four cases in the town of Goleniów and one in the village of Załom; in Przybiernów commune, there was one case in the village of Łoźnica. The age of the cases ranged from 7 to 25 years (Table): in the age group six to 19 years, the incidence rate in the two communes was 78 per 100,000 population. The overall incidence rate in the county was eight per 100,000 population, whereas the annual national incidence of the disease is around one per 100,000 population [2-4]. In Goleniów and Przybiernów communes, the overall incidence rates were 15 per 100,000 population and 19 per 100,000 population, respectively.

Half of the cases were female (Table). No epidemiological links were established between the patients and, to our knowledge, none of the cases were imported.

Three cases presented with meningococcal sepsis along with meningitis, two with meningococcal sepsis and one with meningitis (Table). All patients except the case with meningitis developed petechial rash. Four patients were admitted to a hospital in Goleniów County, where samples were taken and they received initial therapy with third-generation cephalosporins (ceftriaxone or cefotaxime). They were then transported to three regional hospitals in Szczecin (the capital city of West Pomerania), where the two other patients were admitted directly. All the patients recovered fully.

Control of the outbreak

Immediately after diagnosis of possible IMD, based on clinical assessment of symptoms, the cases were reported by the hospitals to the local Sanitary Inspectorate, and chemoprophylaxis (with rifampicin, cefotaxime, ciprofloxacin or azithromycin) was given to the cases' close contacts (Table), as recommended by the National Reference Centre for Bacterial Meningitis [14].

Testing at the National Reference Centre showed that all cases were infected with *N. meningitidis* serogroup C, serotype 2a, serosubtype P1.5,2 of ST-11. Analysis of restriction fragment length polymorphism showed indistinguishable PFGE patterns. DNA sequencing of the *porA* and *fetA* genes revealed that all had PorA variant P1.5,2 and FetA variant F3-3. All isolates were susceptible to penicillin, cefotaxime, chloramphenicol, ciprofloxacin and rifampicin.

On 20 March 2009 (10 days after the first case developed symptoms), a campaign began, organised by the local health authorities to educate hospital physicians, other health professionals and the general public about IMD. Among others things, it included meetings in kindergartens and schools for children and parents, the posting of relevant publications on local and regional governmental and Sanitary Inspectorate websites, publication of articles and notices in local newspapers, television spots and dissemination of special posters and leaflets.

Ten days later, the country's Working Party on Meningococcal Infections, acting as an advisory body to the Chief Sanitary Inspectorate, reviewed the epidemiological situation and strongly recommended vaccination against meningitis to the Ministry of Health. As incidence was highest in the age group six to 19 years, the Ministry of Health decided to vaccinate people in that age group in Goleniów and Przybiernów communes, as well as children in the same age group from other communes who attended schools in the affected region (approximately 6,500 children). Additionally, police, border guards and airport ground staff up to 24 years old from Goleniów County were vaccinated. Each person received one dose of meningococcal conjugated vaccine against serogroup C. Young adults (aged older than 19 years) in the general population were excluded from compulsory vaccination, even though the oldest patient was 25 years old. This was primarily due to

organisational difficulties in reaching this population for vaccination. In addition, as the oldest case in the outbreak had Hodgkin disease, it was thought that this patient may have been immunocompromised, due to chemo- and radiotherapy.

Vaccination began on 9 April 2009 and was scheduled to end on 30 April. However, as some children could not be vaccinated during this time, due to temporary contraindications, and to increase coverage, further vaccination took place from 4 to 29 May 2009. By the end of May, almost 82% (n=5,256) of the target population had been vaccinated. This value was calculated using the number of residents of Goleniów and Przybiernów communes, the number of children aged six to 19 years from other communes who attended schools in the affected area and vaccine uptake. Any children who missed the vaccination in April and May were then scheduled for vaccination from 15 to 19 June 2009.

Patient data and typing results of *Neisseria meningitidis* isolates responsible for invasive meningococcal disease, Goleniów County, Poland, March 2009 (n=6)

Case number	Age (years)	Sex	Date of symptom onset (2009)	Diagnosis	Sample	Serogroup	Multilocus sequence type	PorA variant	FetA variant	Number of contacts who received chemoprophylaxis
1	7	M	10 Mar	Sepsis	Blood	C	11	P1.5,2	F3-3	7
2	17	M	13 Mar	Meningitis	CSF	C	11	P1.5,2	F3-3	37
3	17	F	17 Mar	Meningitis and sepsis	Blood	C	11	P1.5,2	F3-3	3
4	13	F	17 Mar	Meningitis and sepsis	CSF and blood	C	11	P1.5,2	F3-3	6
5	25	M	24 Mar	Meningitis and sepsis	Blood	C	11	P1.5,2	F3-3	3
6	15	F	30 Mar	Sepsis	Blood	C	11	P1.5,2	F3-3	14

CSF: cerebrospinal fluid; F: female; M: male.

FIGURE

Number and percentage of laboratory-confirmed invasive meningococcal disease cases, by mode of identification, Poland, 2006–2009 (n=1,106)[a]
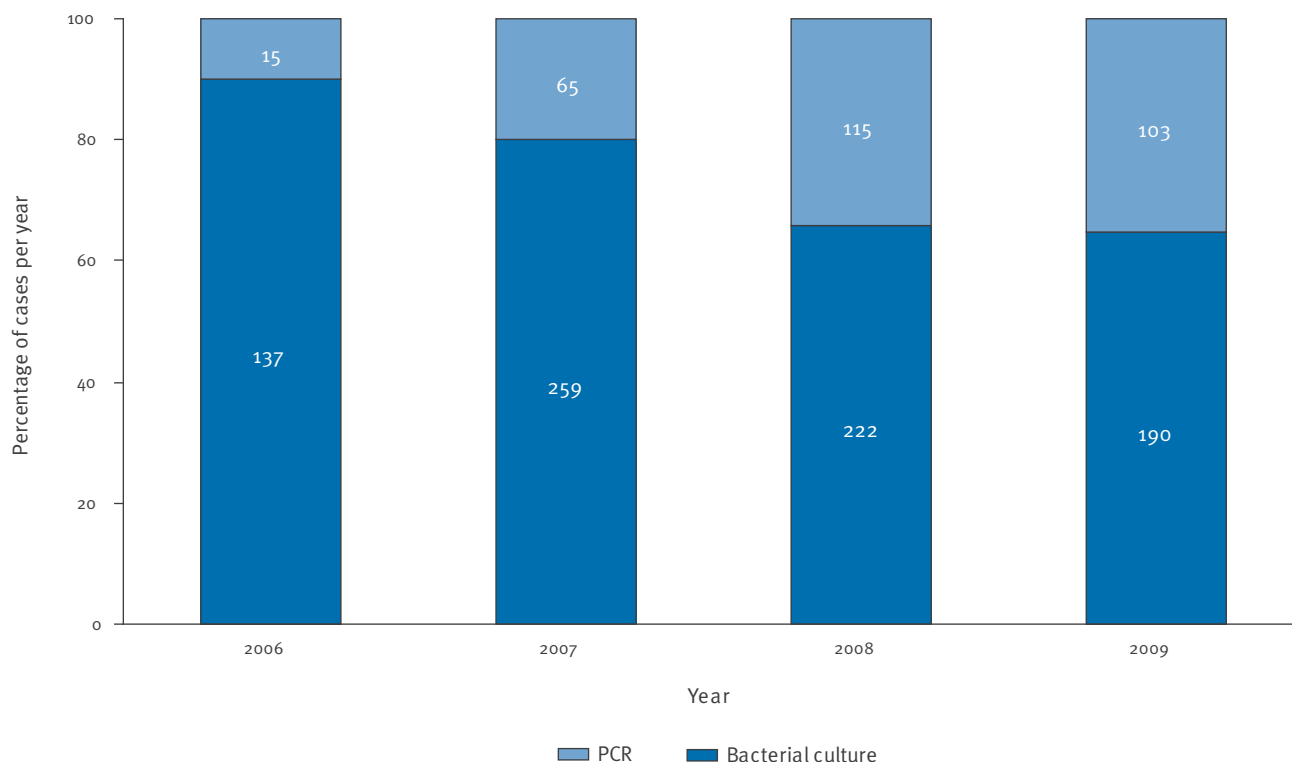
PCR: polymerase chain reaction.
[a] Tested by the National Reference Centre for Bacterial Meningitis.

Discussion

Goleniów County, where the outbreak described took place, is situated in the West Pomeranian region, which had previously experienced a very high case-fatality rate for IMD [6]. At that time, in 2004, the Working Party on Meningococcal Infections had not yet been established, but the situation was closely observed by regional and Chief Sanitary Inspectorates. Despite widely disseminated information and regional alerts directed at health professionals, only 13 of 21 IMD cases were confirmed by bacterial culture and only 12 isolates were available for further testing at the National Reference Centre at that time (in 2004). There was also poor culture confirmation of IMD in 2006–2007, when at least five outbreaks of IMD, also associated with a high case-fatality rate, were notified in different parts of Poland [7,8]. Generally, the number of blood samples sent for microbiological evaluation in Poland is still low, due to limited financial means. According to data collected by the European Antibiotic Resistance Surveillance System (EARSS), the frequency of bacterial culture from blood in Poland is several times lower than that in some other European countries such as the Netherlands, Spain, Sweden and the United Kingdom [15].

As a result, the National Reference Centre for Bacterial Meningitis decided in 2008 to enhance laboratory-based surveillance of invasive bacterial infections, including IMD, by building a voluntary network (called BINet) for monitoring community-acquired invasive bacterial infections, of up to 150 Polish hospitals and microbiological laboratories. For all laboratories in the network, transport of isolates from the hospitals to the National Reference Centre and the tests carried out there are provided free of charge. Two years after BINet started, the education of clinicians and microbiologists and the increase in bacteriological testing have substantially improved the identification of community-acquired invasive bacterial infections in Poland, especially those caused by *N. meningitidis* (Figure) and *Streptococcus pneumoniae* [8,16]. This enhanced monitoring and the earlier experience gained in the West Pomeranian region led to better handling of microbiological specimens and resulted in full characterisation of all isolates from the Goleniów County outbreak. Of the three hospitals that sent isolates to the National Reference Centre, one has not been involved in BINet, but cooperates closely with the National Reference Centre. Due to rapid diagnosis and correct management, all the patients fully recovered, despite having infections caused by hypervirulent ST-11 meningococci, generally associated with a high case-fatality rate [17,18]. This may illustrate that education results in higher awareness of healthcare workers and the general population, enabling immediate reaction when there is probable meningococcal infection.

All cases (n=34) in all the five reported outbreaks in Poland in 2006 and 2007, as well as 56 sporadic cases notified during this time, were infected with an ST-11 meningococcus that had a PorA variant (P1.5-1,10-1) differing from the PorA of ST-11 isolates identified in Poland from 1997 to 2005 (P1.5,2) [7]. The meningococci responsible for the Goleniów County outbreak had the same PorA variant as that of ST-11 meningococci isolated before 2006 (P1.5,2). However, the Goleniów County isolates had an FetA variant (F3-3) that differed from all sequenced ST-11 isolates responsible for IMD cases before 2009 in Poland (generally F3-6, only two isolates had F3-9) (unpublished data). It has been suggested that even very small alterations in antigenic characteristics, which can be the result of a point mutation, may result in an increase in the number of IMD cases in particular areas [19,20]. Such antigenic changes were observed in the PorA and FetA proteins of ST-11 isolates before the 2006 and 2007 outbreaks in Poland and in the 2009 Goleniów County outbreak described here, which may partially explain the occurrence of these outbreaks.

In spite of increases in the number of IMD sporadic cases and outbreaks caused by serogroup C meningococci over the last few years, Poland still does not carry out mass vaccination against this serogroup. The national health budget is very limited, evidenced by the fact that Poland only introduced mass vaccination against *Haemophilus influenzae* type b (Hib) in 2007. Consequently, when meningococcal outbreaks occur, the situation is thoroughly analysed by the Working Party on Meningococcal Infections, which may recommend obligatory vaccination to the Ministry of Health when there is an epidemiological threat.

Despite limited resources, more and more parents and local authorities are deciding to vaccinate their children and their citizens (in certain age groups) respectively against serogroup C meningococci. In 2008, more than 110,000 doses of vaccine against meningococci C were sold in Poland [21]. Vaccination initiatives should be considered across the country, including in Goleniów County, where at the end of August 2009, a non-vaccinated two-year-old boy developed fatal septicaemia caused by meningococci of serogroup C, ST-11 (with the same variants of PorA and FetA as the Goleniów County outbreak isolates) (unpublished data).

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