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Confirmed case of Zika virus congenital infection, Spain, March 2016

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We describe Zika virus (ZIKV) vertical transmission in an imported case in Spain, in a 17-week pregnant woman. ZIKV IgG, IgM and RNA were detected in serum in week 17. At 19 weeks, ultrasound scan revealed fetal malformations and ZIKV was detected in the amniotic fluid. Pregnancy was terminated at week 21; autopsy of the fetus revealed bilateral hydrocephalus, brain microcalcifications and arthrogryposis multiplex congenita. ZIKV was detected in the umbilical cord and brain tissue.

Case description

A pregnant woman in her mid-twenties (week 17 of pregnancy), from Venezuela, was admitted to the Gynaecology Department of a hospital in Spain, for routine follow up, in March 2016, while she was visiting Spain. Her medical history was uneventful apart from a generalised skin rash in January 2016, at eight weeks of gestational age. This led us to investigate a ZIKV infection, given the epidemiological situation in Venezuela. The rash had lasted for 48 hours but there was no microbiological diagnosis. At 12 weeks of gestational age, the ultrasound scan was normal, and the results of laboratory tests for HIV, *Treponema pallidum* and *Toxoplasma gondii* were negative.

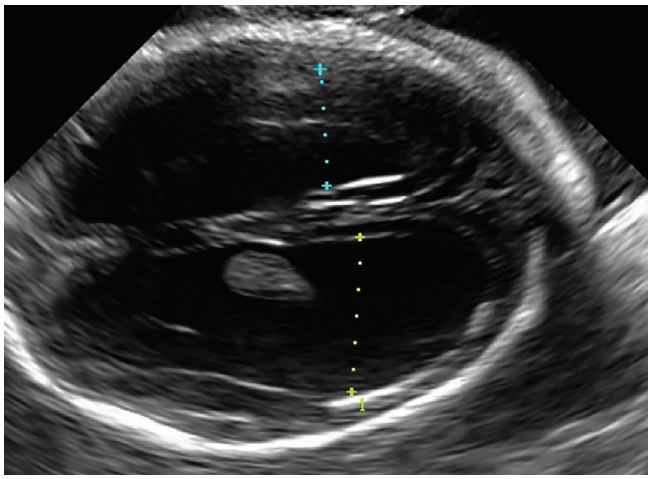
Serum samples were collected upon hospital admission in March and recent ZIKV infection was diagnosed by serology and molecular biology. ZIKV IgG and IgM antibodies were detected by indirect immune fluorescence test (Euroimmun Arboviral Fever Mosaic 2, Luebeck, Germany). ZIKV IgG antibodies were confirmed by plaque reduction neutralization test (PRNT). NS2A protein gen of ZIKV was detected (2.4 x104 copies/mL) by real-time quantitative RT-PCR using Light Mix Modular ZikaVirus (Tib Molbiol, Berlin, Germany) and Light Cycler Multiplex RNA Virus Master (Roche Diagnostics, Mannheim, Germany) in a Cobas Z 480 analyser (Roche Diagnostics, Indianapolis, United States). Extraction of nucleic acids was performed with the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics, Indianapolis, United States). Ultrasound scan at 19 weeks of gestational age showed fetal hydrocephalus with dilation of both lateral ventricles (Figure 1), and stiffness and severe contractures of the four extremities compatible with arthrogryposis multiplex congenita (AMC) (Figure 2).

Amniotic fluid was obtained by amniocentesis. Chromosomal abnormalities were discarded by array comparative genomic hybridisation and karyotype analysis. ZIKV RNA was detected in amniotic fluid (9.1 x104 copies/mL) by real-time quantitative RT-PCR. Toxoplasma gondii, rubella virus, cytomegalovirus, herpes virus, erythrovirus B19 and measles virus infections were discarded by PCR in amniotic fluid.

Due to severe malformations and brain disease, the neonatal health prognosis was poor. The patient asked for voluntary termination of the pregnancy, and the procedure was approved by national and hospital ethics committees. Medical termination of the pregnancy was performed at 21 weeks of gestation. Autopsy of the female fetus (295 g) showed no microcephaly (cranial, thoracic and abdominal circumference were 17 cm, 16 cm and 14 cm respectively, normal for sex and gestational age) but confirmed AMC with flexion contracture and deformity of joints of all four limbs, extreme

Figure 1

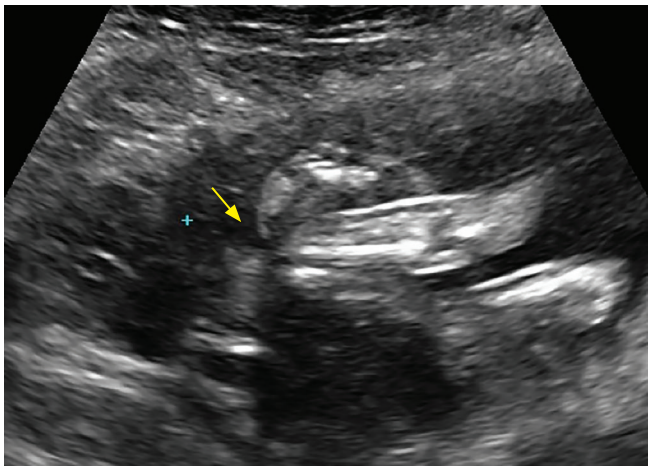
Ultrasound scan on fetal skull showing bilateral hydrocephalus, case of vertical transmission of Zika virus infection, gestation week 19, imported to Spain, March 2016



Ventricular measurements 14.3 mm (yellow) and 10.7 mm (blue) (norm: ≤10 mm)

Figure 2

Ultrasound scan on fetal extremities, case of vertical transmission of Zika virus infection, gestation week 19, imported to Spain, March 2016



The arrow indicates severe contracture and deformation with extreme bending and twisting of the wrist.

flexion of hips and crossed femurs. Under-developed muscles with replacement of muscle by adipose tissue and fibrous proliferation in interarticularis spaces were also found. In addition, fetal hydrocephalus with dilation of both lateral ventricles, cerebral cortex 2.5 mm thick and multiple calcifications at cortical level and brainstem were diagnosed (Figure 3).

ZIKV real-time quantitative RT-PCR was performed in placenta, umbilical cord and brain tissue samples, as previously described. Before nucleic acid extraction, ca 10 mg of tissue were treated with 50 µL of proteinase K at 56 °C until sample digestion, and then heated at 95 °C for 10 min to inactivate proteinase K. ZIKV RNA was detected in umbilical cord (threshold cycle, Ct: 36.7) and brain tissue (Ct: 22.1), but it was not detected in the placenta sample. For phylogenetic analysis, amplification of NS5 gene (192 bp) was performed from amniotic fluid sample as previously described [1] with Mega 7 Software and it was deposited in GenBank (accession number: KX358623). The sequence clustered within the Asian lineage.

Background

ZIKV is an arbovirus (arthropod-borne virus) of the genus Flavivirus. It was isolated for the first time in 1947 from the blood of a Rhesus monkey in the Zika forest (Uganda), but the infection was relatively unknown until the recent outbreak in South America. Prior to 2015, minor ZIKV outbreaks were reported in areas of Africa, south-east Asia and the Pacific Islands [2]. In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed ZIKV infections in Brazil [3], which quickly spread all over the South America continent.

Discussion

Local mosquito transmission of ZIKV infection was reported in Venezuela in November 2015 [4]. In January 2016, a total of 252 cases of Guillain-Barré syndrome with a spatiotemporal association to Zika virus were reported. Zika virus infection was confirmed in three of them [5].

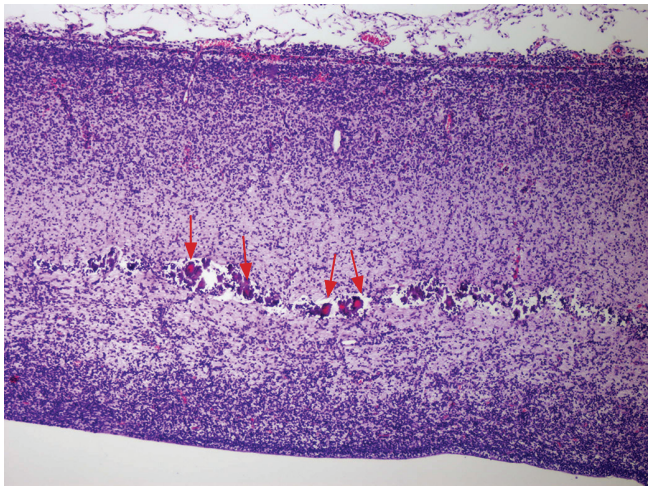
Phylogenetic analysis based on the conserved region NS5 has revealed the existence of two major lineages: the African lineage that includes the African strains and the Asian lineage which has recently emerged in the Pacific and the Americas including the Asian and American strains [6]. In this case, phylogenetic analysis showed that ZIKV strain isolated from this patient belonged to the Asian lineage.

The illness caused by ZIKV, commonly named Zika virus disease (ZVD), usually has a benign cause and presents with signs and symptoms similar to other arbovirus infections such as dengue virus (DENV) and chikungunya virus (CHKV): mild fever, exanthema, arthralgia and/or non-purulent conjunctivitis. Recently, ZIKV infection has been associated with neurological complications, such as Guillain–Barré syndrome, but encephalitis or meningitis have also been reported [7]. The patient reported generalised skin rash compatible with ZIKV infection during her stay in Venezuela.

ZIKV is transmitted through mosquito bites of the genus *Aedes*, mainly *Aedes aegypti,* which is the most important vector in Asia and the Americas and in some regions in the Pacific. Other possible modes for human infection include sexual transmission and blood transfusion, and vertical transmission from mother to fetus can have serious consequences. If a pregnant woman

Figure 3

Microphotography of the entire cerebral cortex thickness with abundant microcalcifications, case of vertical transmission of Zika virus infection, gestation week 21, imported to Spain, April 2016



Arrows indicate microcalcifications.

acquires a ZIKV infection, the virus might cross placental barrier causing congenital infection. In this case, ZIKV RNA was detected in amniotic fluid, confirming that ZIKV crossed the placental barrier. When this happens, the fetus might develop brain damage including microcephaly and, less frequently, calcifications, ventriculomegaly or hydrocephalus, and other congenital malformations such as arthrogryposis [8].

Viraemia of ZIKV infection is relatively short, and viral RNA is usually detected in serum samples only around seven days after onset of symptoms. It is remarkable that in this case, ZIKV RNA was detected in the serum of the pregnant woman up to two months after the acute phase of the disease. Persistent viraemia was previously described in another case of congenital Zika transmission and it might be a consequence of high viral replication in the fetus [9].

The recent outbreaks in Brazil and French Polynesia indicate that the greatest risk of brain damage for the fetus is in the first trimester, often between seven and 13 weeks of gestation [10,11]. A preliminary report from Brazil indicated that fetal abnormalities were present in almost 30% of women with ZIKV infection during pregnancy [12]. In the case presented here, microcephaly was not present in the ultrasound scan at 19 weeks of gestation, probably due to the short gestational age, and this is in agreement with other reported cases in which microcephaly was not diagnosed until 27 to 35 weeks [12]. However, other malformations, such as hydrocephalus and arthrogryposis, were detected in this case. Presence of ZIKV RNA was detected in the umbilical cord and brain tissue but not in placenta as recently reported in another congenital case [13]. Another study showed that ZIKV is unlikely to access the fetal compartment by its direct replication in placental tissue. This might be explained due to the potent antiviral properties of type III interferons (IFNs), specifically IFNλ1, which protects the human placental trophoblasts from viral infections, suggesting that ZIKV may invade the intrauterine cavity by unknown mechanisms that are independent of direct placental infection [14]. In this case, comparison of the Ct values between umbilical cord and brain tissue indicates a higher viral load (ca 10,000 times higher) in the central nervous system (CNS), showing a strong neurotropism of the virus, although the mechanism is not clear. A possible persistence of ZIKV in the fetal brain because of the immunologically secure milieu for the virus was suggested [15]. Additionally, recent studies found that neural progenitor cells are more susceptible to ZIKV infection than mature cortical neurons, explaining microcephaly and other abnormalities in the developing brain [16-18].

In the Zika situation report of the World Health Organization (WHO) on 6 June 2016 [19], only eleven countries or territories have reported microcephaly and/or CNS malformation cases potentially associated with ZIKV infection. The total number of cases reached 1,520, and most of them are related to the recent outbreak in Brazil. In Spain, the Ministry of Health reported 141 cases of confirmed ZIKV infection on 6 June 2016, all of them imported from areas with active transmission [20]. Nineteen cases were pregnant women, but no evidence of intrauterine transmission was detected, except in this case. The first case of imported ZIKV infection and two cases of ZIKV infection in pregnant women in Spain, have been published recently [21,22]. Aside from this case, there has been only one other confirmed ZIKV congenital infection in Europe [15].

This case highlights the new challenge gynaecologists face when performing ultrasound in pregnant women originating from or having stayed in countries with risk of transmission of Zika. Nowadays, there is a considerable number of pregnant women who travel from South America; moreover, other pregnant women or their sexual partners may travel to affected areas. This may have an impact on prenatal care in Europe. Positive and negative predictive values of screening for Zika virus in amniotic fluid or maternal serum have not yet been established. Accurate and quick detection of the presence of Zika virus in maternal samples in collaboration with expert gynaecologists follow-up might help the early diagnosis of congenital Zika infections. It is important to carefully communicate risks associated with ZVD to those possibly concerned, and to improve the evidence base to perform well-informed risk assessments.

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Conflict of interest

None declared.

Authors' contributions

Wrote the manuscript: SP, RT, AL, OR; performed laboratory investigations: SP, RT, JJC, OR, AC, MPSS, AV, RC; revised the manuscript: JJC, EP, AC, MPSS, AP, AV, RC, CQ; managed the patient: AL, OR, EP, AP, CQ.

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Influenza A(H1N1)pdm09 virus exhibiting enhanced cross-resistance to oseltamivir and peramivir due to a dual H275Y/G147R substitution, Japan, March 2016

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An influenza A(H1N1)pdm09 virus carrying a G147R substitution in combination with an H275Y substitution in the neuraminidase protein, which confers cross-resistance to oseltamivir and peramivir, was detected from an immunocompromised inpatient in Japan, March 2016. This dual H275Y/G147R mutant virus exhibited enhanced cross-resistance to both drugs compared with the single H275Y mutant virus and reduced susceptibility to zanamivir, although it showed normal inhibition by laninamivir.

Detection of a dual H275Y/G147R mutant influenza A(H1N1)pdm09 virus

In the context of our nationwide monitoring for antiviral-resistant viruses, we previously reported that a large community cluster of influenza A(H1N1)pdm09 virus exhibiting cross-resistance to oseltamivir and peramivir had occurred in Hokkaido, Japan between November 2013 and February 2014 [1,2]. Of a total of 2,531 A(H1N1)pdm09 viruses investigated in the 2013/14 influenza season, 105 (4.1%) were shown to harbour the H275Y substitution in the neuraminidase (NA) protein. In the 2015/16 season (Figure 1), we screened 1,938 A(H1N1)pdm09 viruses by allelic discrimination [3] and detected 39 (2.0%) H275Y mutant viruses.

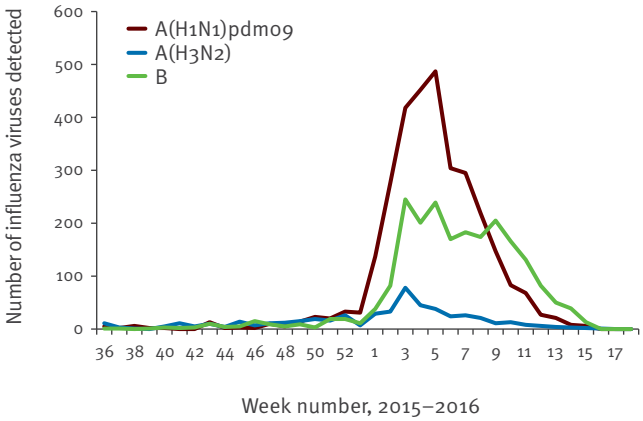
No epidemiological links were identified among the patients infected with the H275Y mutant viruses except for five nosocomial infections. Among 34 sporadic cases, 11 (32%) cases had no exposure to NA inhibitors before the specimen collection. This is consistent with our previous study that 19 (32%) of 59 sporadic cases had no exposure to NA inhibitors in the 2013/14 season [2]. One H275Y mutant virus with an additional G147R substitution in the NA protein was detected from a sporadic case treated with peramivir in March 2016. Both substitutions were confirmed in the corresponding clinical specimen. Deep sequencing analysis of the specimen using MiSeq (Illumina, California, United States) revealed that the G147R substitution was detected in 21% of mixed sequence populations with 147G wild type, in contrast to H275Y, which was detected at a rate of 100%. The results suggested that the H275Y mutant virus had acquired the additional G147R substitution in the patient during peramivir administration. The NA H275Y mutation of the A(H1N1)pdm09 and the former seasonal A(H1N1) viruses confers cross-resistance to oseltamivir and peramivir [4] and the NA G147R is linked to slightly reduced susceptibility of the highly pathogenic avian A(H5N1) virus to oseltamivir and zanamivir [5]. Searching the EpiFlu Database of the Global Initiative on Sharing All Influenza Data (GISAID) yielded 18,172 A(H1N1)pdm09 viruses, of which nine were G147R mutant viruses (Table). No dual H275Y/G147R substitution appears to have been previously reported however.

Clinical course of the patient infected with the dual H275Y/G147R mutant virus

The patient, a woman in her early 50s with malignant lymphoma who was receiving chemotherapy, was hospitalised with myelosuppression in late February 2016. She received prophylaxis with laninamivir (40 mg) on the same day because her husband had been diagnosed as having influenza A virus infection. Three days later, she had onset of illness and tested positive for influenza A. At this time, peramivir was administered intravenously at a dosage of 600 mg daily for three intermittent periods of 5 days because of persistent influenza A virus infection. She developed left lower

Figure 1

Detection of influenza viruses, September 2015–April 2016 (week 36, 2015–18, 2016)[a], Japan (n = 5,778)



Weekly reports of influenza virus isolation/detection by the National Epidemiological Surveillance of Infectious Diseases.

a Week 36 started on 31 August 2015.

lobe pneumonia 20 days post-disease onset and alveolar haemorrhage 12 days after, but no bacterial pathogens were isolated from sputum or blood samples. She died 5 days later. A nasal swab specimen of the patient was collected only at the end of the second intermittent administration of peramivir and an influenza A(H1N1)pdm09 strain A/Hiroshima/13/2016 was isolated in Madin-Darby canine kidney (MDCK) cells from the specimen (Table). We could not obtain a specimen from the husband.

Antiviral susceptibility of the dual H275Y/G147R mutant virus

After isolation in MDCK cells, A/Hiroshima/13/2016 possessed the H275Y and G147R substitutions in 100% population, respectively, although the G147R mutation was detected at a rate of 21% in the specimen. This result indicates that the virus carrying both substitutions had become predominant during MDCK cell culture.

We compared the susceptibilities of the dual H275Y/G147R mutant virus and single H275Y mutant viruses isolated during the same influenza season to four NA inhibitors approved in Japan: laninamivir, oseltamivir, peramivir and zanamivir (Figure 2).

Oseltamivir carboxylate, peramivir and zanamivir were purchased from Sequoia Research Products (Pangbourne, UK) and laninamivir was kindly provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). The susceptibilities of the viruses to NA inhibitors were determined by fluorescent NA inhibition assay with the NA-Fluor Influenza Neuraminidase Assay Kit (Applied Biosystems, California, United States). Results were expressed as the drug concentrations required to inhibit NA activity by 50% (IC_{50}). To interpret the NA inhibitor susceptibility, we used the World Health Organization criteria, which are based on the fold change of IC_{50} values compared with the median IC_{50} values of the same subtype/lineage [6]. For influenza A viruses, normal (‹10-fold increase of IC_{50} value), reduced (10–100-fold increase) or highly reduced (›100-fold increase) inhibition were defined.

The IC_{50} values of the viruses to laninamivir, oseltamivir, peramivir and zanamivir are shown in Figure 2. The median IC_{50} values of 19 single H275Y mutant viruses to oseltamivir and peramivir were 920- and 260-fold higher, respectively, than those of the 236 wild-type viruses. The dual H275Y/G147R mutant virus exhibited 2,600- and 1,400-fold higher IC_{50} values to oseltamivir and peramivir, respectively, compared with the wild-type viruses. These results indicate that the dual H275Y/G147R mutant virus showed highly reduced inhibition with high increases in oseltamivir and peramivir IC_{50} values compared with values for the single H275Y mutant viruses. Furthermore, the IC_{50} value of the dual mutant virus to zanamivir was ca fivefold higher than the median IC_{50} values of the wild type and the single H275Y mutant viruses, although the dual mutant virus showed normal inhibition by laninamivir.

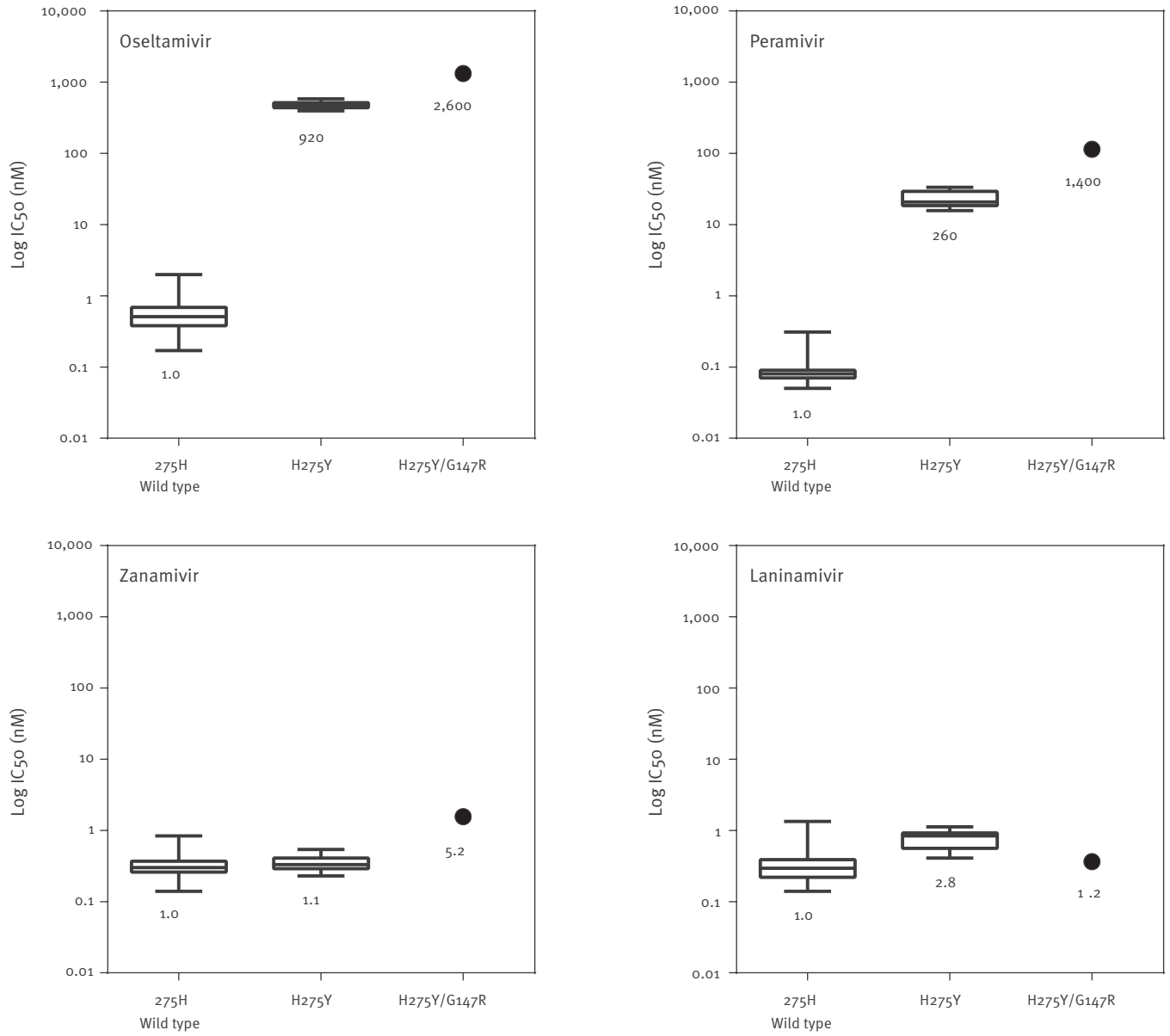
Discussion

The first widespread community cluster of the H275Y mutant A(H1N1)pdm09 virus was detected in Newcastle, Australia in 2011 [7]. The H275Y substitution in the NA protein would destabilise the mutant virus. However, two additional V241I and N369K substitutions in the NA of H275Y mutant viruses were reported to increase their replication and transmission fitness, contributing to efficient transmission [8,9]. Almost all recently circulating A(H1N1)pdm09 viruses possess these permissive substitutions, suggesting an increased risk for H275Y mutant viruses to emerge and spread globally [10]. Indeed, during the 2013/14 influenza season, the H275Y mutant viruses from a large community cluster in Hokkaido, Japan carried these permissive substitutions. Following this finding, we subsequently increased nationwide monitoring for the H275Y mutant viruses in the 2015/16 season and detected an H275Y mutant with V241I and N369K and an additional G147R substitution in the NA protein from an immunocompromised inpatient. The IC_{50} fold changes of a number of NA inhibitors for the dual H275Y/G147R mutant virus compared with those for the single H275Y viruses showed clearly the synergistic effect of this dual substitution (Figure 2).

Hooper et al. reported that the G147R substitution in the NA protein has been detected in A(H1N1)pdm09, in the former seasonal A(H1N1) as well as in the A(H5N1) viruses where it conferred receptor-binding activity to the NA proteins of these viruses, similar to a D151G substitution in the NA of A(H3N2) viruses [11,12]. Phylogenetic analyses suggested that these G147R mutant viruses occurred sporadically in nature, while

Figure 2

Susceptibility to neuraminidase inhibitors of influenza A(H1N1)pdm09 viruses with H275Y and G147R substitutions detected in Japan, September 2015–April 2016 (n = 256)



IC50: 50% inhibitory concentration.

The IC50 values of the viruses to laninamivir, oseltamivir, peramivir and zanamivir were determined by fluorescent neuraminidase inhibition assay. Box-and-whisker plots of the IC50 values (medians and interquartile ranges) are shown. The numbers at the bottom of each box-and-whisker plot indicate the fold change in IC50 values compared with the median IC50 values of 275H wild-type viruses.

the D151G substitution emerged during propagation of virus in MDCK cell culture [11,12].

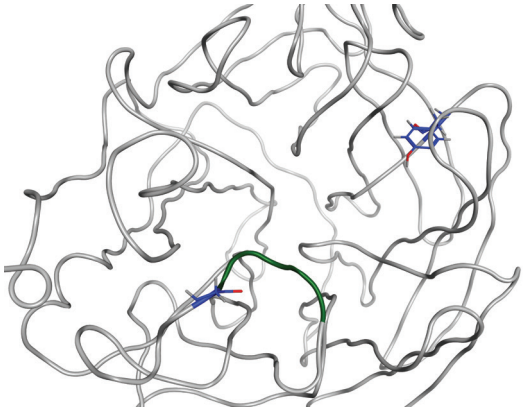
Residue 147 is located in a 150-loop that includes residues 147 to 152 adjacent to the NA active site as shown in Figure 3 [13]. A previous study reported it as having an essential role in the conformation of the 150-loop [14]. Our structural analysis of the NA protein of the dual H275Y/G147R mutant virus using Molecular Operating Environment, MOE, (Chemical Computing Group Inc., Quebec, Canada) [15] suggests that the G147R substitution may alter the stability of the 150-loop because the side chain of arginine is larger than that of glycine (Figure 3), negatively affecting the binding affinity to NA inhibitors.

The G147R substitution of N1 NA has been shown to slightly decrease enzymatic activity but not to affect the viral replication fitness [12]. These results, together with the findings of recent H275Y mutant viruses carrying permissive substitutions, V241I and N369K, suggest that the dual H275Y/G147R mutant virus had the

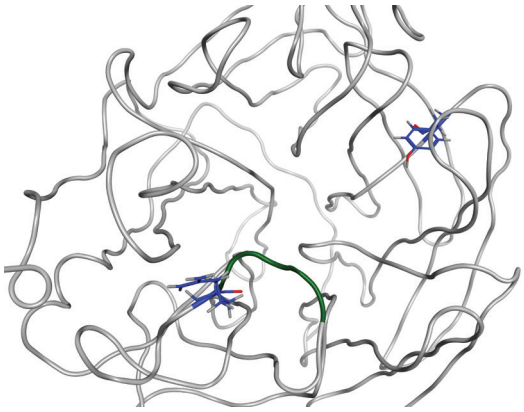
Figure 3

Three-dimensional structure of the neuraminidase protein of influenza A(H1N1)pdm09 virus with the H275Y and G147R substitutions

A. H275Y



B. H275Y/G147R



Structure models of the neuraminidase proteins of the single H275Y (A) and the dual H275Y/G147R (B) mutant viruses were constructed by homology modelling. The crystal structure of the A(H1N1)pdm09 neuraminidase protein (PDB ID: 4B7R) was used as the modelling template.

A 150-loop in the neuraminidase protein is shown in green.

potential to replicate efficiently. In this study, we found that the dual H275Y/G147R mutant virus grew well in cell culture. Furthermore, the patient infected with the dual H275Y/G147R mutant virus developed pneumonia without isolation of bacterial pathogens, suggesting viral pneumonia with this dual mutant virus.

Immunocompromised patients are at great risk for emergence of the antiviral resistant virus because of the selective pressure from prolonged exposure to antiviral drugs [16]. A high rate and prolonged shedding of the H275Y mutant A(H1N1)pdm09 virus in immunocompromised patients treated with oseltamivir and/or peramivir were reported previously [17]. As a specimen from the husband of the patient was unavailable, we cannot rule out that the patient was infected with a virus readily carrying the G147R substitution. Results of this study nevertheless suggest that this substitution likely occurred in the patient during peramivir treatment. On the other hand, whether the H275Y mutation had already occurred or not before infection remains unclear. Other additional substitutions, I223R and S247N, in the NA protein of H275Y mutant A(H1N1)pdm09 viruses have been reported and showed a synergistic effect with the H275Y substitution on the reduction of NA inhibitor susceptibility [18,19]. Although the frequencies of these dual substitutions were low, the surveillance of antiviral-resistant viruses should be continued to protect public health and support clinical management, particularly for high risk populations.

The Influenza Virus Surveillance Group of Japan

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Table

Influenza A(H1N1)pdm09 viruses with G147R substitution submitted to the GISAID's EpiFlu Database

GISAID Isolate ID	Country	Collection date	Isolate name	Originating laboratory	Submitting laboratory	NA substitution	
EPI_ISL_63723	Finland	2009–07–24	A/Finland/614/2009	Other Database Import		G147R	275H
EPI_ISL_108540	China	2011–01–08	A/Tianjinhedong/SWL44/2011	Other Database Import		G147R	275H
EPI_ISL_123113	Singapore	2011–02–09	A/Singapore/SGH02/2011	Other Database Import		G147R	275H
EPI_ISL_98830	Argentina	2011–08–05	A/Argentina/656/2011	Instituto Nacional de Enfermedades Infecciosas	Centers for Disease Control and Prevention	G147R	275H
EPI_ISL_150164	India	2013–09–07	A/India/3743/2013	National Institute of Virology	Centers for Disease Control and Prevention	G147R	275H
EPI_ISL_212270	UK	2014–03–13	A/England/354/2014	Microbiology Services Colindale, Public Health England	Microbiology Services Colindale, Public Health England	G147R	275H
EPI_ISL_164370	India	2014–05–24	A/India/5964/2014	National Institute of Virology	Centers for Disease Control and Prevention	G147R	275H
EPI_ISL_207955	United States	2016–01–01	A/Wisconsin/02/2016	Wisconsin State Laboratory of Hygiene	Centers for Disease Control and Prevention	G147R	275H
EPI_ISL_209086	Russian Federation	2016–01–15	A/Orenburg/05/2016	Information not available	State Research Center of Virology and Biotechnology Vector	G147R	275H
EPI_ISL_220376	Japan	2016–03–11	A/Hiroshima/13/2016	Hiroshima Prefectural Technology Research Institute	National Institute of Infectious Diseases	G147R	H275Y

GISAID: Global Initiative on Sharing All Influenza Data; NA: neuraminidase.

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Conflict of interest

None declared.

Authors' contributions

Designed the analyses: ET, SF, SW, TO. Analysed and interpreted data: ET, SF, MS, KN, NK, TK, YS, TS, SW, TO, The members of the Influenza Virus Surveillance Group of Japan. Drafted the article: ET. Revised the article: SW, TO.

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Clinical characteristics and public health management of invasive meningococcal group W disease in the East Midlands region of England, United Kingdom, 2011 to 2013

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In England and Wales, meningococcal disease caused by group W has historically been associated with outbreaks of disease among travellers to high-risk countries. Following a large outbreak associated with travel to the Hajj in 2000, the number of cases declined and, in 2008, only 19 laboratory-confirmed cases were identified nationally. In 2013, in the East Midlands region of England, eight cases of meningococcal disease caused by this serogroup were recorded, compared with six from 2011 to 2012. To explore this further, data for all cases with a date of onset between 1 January 2011 and 31 December 2013 were collected. Data collected included geographical location, clinical presentation and outcome. Fourteen cases were identified; two died as a result of their illness and two developed long-term health problems. No commonality in terms of geographical location, shared space or activities was identified, suggesting that group W is circulating endemically with local transmission. Clinical presentation was variable. Half presented with symptoms not typical of a classical meningococcal disease, including two cases of cellulitis, which may have implications for clinicians, in terms of timely identification and treatment, and public health specialists, for offering timely antibiotic chemoprophylaxis to close contacts.

Introduction

There are 13 *Neisseria meningitidis* capsular groups known to cause invasive meningococcal disease (IMD); in England and Wales, around 80% of cases are caused by capsular group B (MenB) [1]. Meningococcal group W (MenW) has historically been responsible for only a small proportion of disease in England and Wales. In 2000, however, there was a major international outbreak of meningococcal disease caused by group W (type 2a subtype P1.2, P1.5) associated with pilgrims attending the Hajj. Previously, this strain had only rarely been isolated in European countries [2]. In just over four months in 2000, 90 cases were reported from nine European counties, with 42 of these in the United Kingdom (UK) and 24 in France [2].

Following the Hajj-related outbreak, MenW became an increasingly common cause of IMD in different parts of the world. In Gauteng Province in South Africa (which includes the cities of Pretoria and Johannesberg), for example, MenW is now considered endemic, accounting for 75% of cases in 2005 compared with 7% in 2000 [3]. In 2002, in Burkina Faso, 13,000 suspected cases were recorded, with the highest attack rates seen in infants aged one year and under (1,092/100,000) [4]. Other sub-Saharan countries have similarly reported an increase in MenW disease, also in young children, following the successful implementation of the MenA conjugate vaccine [5,6].

Following the introduction of mandatory vaccination for Hajj pilgrims, the number of MenW cases in England and Wales declined rapidly, from 5.2% (127/2,448) laboratory-confirmed IMD cases in the 2000/01 epidemiological year to1.8% (21/1,164) in 2008/09. Since 2009/10, however, MenW cases have increased year-on-year, with 98 cases confirmed in 2013/14, accounting for 15% of 664 IMD cases [7].

In 2013, health protection teams working in the East Midlands area of England were notified of eight MenW cases, including six cases notified within a three-month period (September to November 2013). We therefore undertook detailed analysis of all laboratory-confirmed

Table 1

Clinical presentation, sub-type, outcome and underlying health conditions by age group for invasive meningococcal group W disease cases observed in the East Midlands region of England, United Kingdom, 2011–2013 (N = 14)

Clinical characteristic	Number of cases by age category			Total (N = 14)
	0–5 years (n = 3)	6–50 years (n = 5)	>50 years (n = 6)	
Recorded symptoms on admission				
Fever	2	1	2	5
Respiratory				
Runny nose	2	0	1	3
Breathing difficulties	1	0	1	2
Chest pain	0	1	1	2
Gastrointestinal symptoms				
Diarrhoea	1	1	1	3
Vomiting	0	1	1	2
Abdominal pain	0	1	0	1
Skin and soft tissue				
Non-blanching rash	1	0	1	2
Cellulitis	0	1	1	2
Bone and joint				
Joint pain	0	0	1	1
Septic joint	1	0	0	1
Neurological				
Headache	0	0	1	1
Non-specific				
Tiredness	0	0	1	1
Underlying health conditions				
Immunocompromised	0	1	0	1
Diabetes	0	0	1	1
Outcome				
Death	1	0	1	2
Serious long-term consequences	0	1[a]	0	1
Other long-term consequences	0	1[b]	0	1
No complications	2	3	5	10

[a] Locked-in syndrome.
[b] Bilateral hearing loss.

MenW cases in the East Midlands over three calendar years (2011–2013) to identify any epidemiological links between the cases and to describe key features, including patient demographics, travel history, co-morbidities, clinical presentation, complications at hospital discharge and outcome.

Methods

All laboratory-confirmed IMD cases reported to Public Health England (PHE) between 1 January 2011 and 31 December 2013 in the East Midlands region of England were identified through a systematic search of HPZone, a national computerised system used to record all health protection activity undertaken by Public Health England. It is designed to assist with public health management of individual cases and to facilitate outbreak and incident management. Data for one area of the East Midlands (Northamptonshire) were only available up to April 2013 as changes in geographical boundaries meant this area became the responsibility of another regional health protection team. Queries were developed to identify any HPZone cases with 'W' and 'meningococcal' mentioned in key recording fields. The confirmed meningococcal cases in the period of interest were also hand-searched. For validation purposes, cases occurring in 2013 were checked against data held by PHE's national Meningococcal Reference Unit (MRU) in Manchester. All identified cases were included in the MRU dataset and no further eligible cases were found. Submission of invasive meningococcal isolates to the MRU allows both phenotypic and comprehensive genotypic characterisation for local and national surveillance, including identification of closely-related strains in clusters and outbreaks. Since July 2010, all clinical isolates have been subjected to whole genome sequencing [8]. The MRU also provides a free national PCR-testing service for National Health Service (NHS) for non-culture confirmation of IMD in

suspected cases and identification of the responsible meningococcal capsular group. Information was collated on method of case identification, the phenotype (serotype and sero-subtype) and the multilocus sequence type (MLST) clonal complex (CC) as described in the Meningitis Research Foundation (MRF) Meningococcus Genome Library MGL, (http://www.meningitis.org/research/genome).

Data from identified MenW cases were extracted using a pre-defined questionnaire and entered into Microsoft Excel for descriptive analysis. Data collected included demographic information, risk factors, activity in the seven days before onset, clinical presentation and outcome. Date of onset, date of hospitalisation and date of notification to PHE were also collected and used to assess any differences in time to notification for MenW cases with typical and atypical clinical presentations. The i2 Analyst's Notebook software package was also used to provide a visual representation of each case and to facilitate the identification of any commonality between cases.

Results

The HPZone search identified 14 laboratory-confirmed MenW cases in the East Midlands region during 2011–13. Just over half of cases were female (n = 8, 57.1%) and only one case had a recorded ethnicity that was not White British. The median age of cases was 47 years (range: 3 months to 87 years) and half the cases were diagnosed in ≥ 45 year-olds. Three cases were in infants (aged < 1 year), including one who died as a result of the infection. All cases were assessed for commonality in relation to date of onset, geographical location and contact history in the seven days before onset. There were six cases in 2 years: three in 2011 and three in 2012, and eight cases in 2013, including six in the period from 1 September 2013 to 30 November 2013. No commonality between these cases could be identified. In terms of activity in the seven days before IMD onset, three had a travel history recorded: one within the UK and two abroad, but not to countries at high-risk for MenW (for example, Sub-Saharan Africa). From the information available, none of the cases had been in contact with anyone who had recently travelled to a high-risk country.

The age distribution, clinical presentation, co-morbidities and outcomes are summarised in Table 1.

The most commonly recorded symptom at admission was fever, followed by coryzal symptoms and diarrhoea. Of the 14 cases, seven were considered to have an atypical presentation. One case presented with abdominal pain and fever and was initially diagnosed with appendicitis, while another case presented with diarrhoea and vomiting only. Two other cases presented with cellulitis and another with a septic joint. The overall median time from hospitalisation to reporting the case to PHE was two days (interquartile range: 2.5 days). For cases with atypical presentation the median was three days (interquartile range: 5 days) compared with 0.5 days (interquartile range: 1.75 days) for those with typical presentation.

Three cases had a known underlying health condition; one was immunocompromised, another had diabetes and the third had borderline myeloma but was not immunocompromised. Two patients died as a result of their illness, an infant and an older adult aged > 85 years. One case also developed locked-in syndrome after reaching hospital in a critical condition and another developed bilateral hearing loss following MenW meningitis.

Thirteen cases were confirmed by culture and one by PCR only (Table 2).

All 13 meningococcal isolates underwent serological phenotyping and 10 (76.9%) were serotype 2a, of which seven (53.8%) were sero-subtypes P1.5 or P1.2 (Table 2).

MLST CC was available for all 13 culture positive cases and of these 10 belonged to clonal complex cc11, thereby confirming the strong association between serotype 2a and cc11. All seven case isolates phenotyped as sero-subtype P1.5 and P1.2 were confirmed as porAVR1 5 and porAVR2 2. The 10 cc11 case isolates were subjected to whole genome sequencing as part of enhanced national surveillance and belonged to the South American/UK strain recently identified and reported by PHE MRU [8]. The PCR-only case was determined to be porAVR1 5 and porAVR2 2 by specific gene sequencing.

Discussion

The recent increase in MenW cases diagnosed in the East Midlands mirrors the national year-on-year rise in laboratory-confirmed MenW cases since 2009 [7]. Detailed analysis of the 14 identified cases found no clear epidemiological link between the cases, but highlighted the severe course of illness with unfavourable outcomes across all age groups. The lack of overseas travel to high-risk countries and a lack of shared activities or space, suggests that this strain is circulating in our region with local transmission. Furthermore, molecular characterisation and whole genome sequencing of clinical isolates found a single strain to be responsible for 10 of the 13 cases [8]. Because of the small number of cases, it is not possible to determine whether this particular strain was associated with more severe disease outcomes, in terms of clinical presentation, long-term complications or death. However, our findings do fit with existing evidence that, when compared with the more common MenB cases, MenW cases were more likely to have atypical clinical presentations [7]. Atypical clinical presentations have important implications in terms of delays in diagnosis and notification to health protection teams, leading to delays in treatment, contact tracing and timely administration of antibiotic

Table 2

Phenotype and genotype of culture for all 14 cases observed in the East Midlands region of England, United Kingdom, 2011–2013

Case number	Age category (years old)	Phenotype	Sequence typing from whole genome sequencing			
			*por*AVR1	*por*AVR2	Sequence type	Clonal complex
1	0–5	W:2a:P1.5,2	5	2	11	11
2	0–5	W (PCR only)	5	2	NA	NA
3	0–5	W:NT:P1.5	5–1	10–4	184	22
4	6–50	W:2a:P1.5,2	5	2	11	11
5	6–50	W:2a:P1.5,2	5	2	11	11
6	6–50	W:2a:P1.2	5	2	11	11
7	6–50	W:2a:P1.5,2	5	2	11	11
8	6–50	W:2a:NT	5	2	11	11
9	>50	W:NT:P1.3	18–1	3	1281	22
10	>50	W:2a:P1.5,2[1]	5	2	11	11
11	>50	W:2a:P1.14	7–2	14	11	11
12	>50	W:2a:P1.5,2	5	2	10651	11
13	>50	W:2a:P1.5,2	5	2	11	11
14	>50	W:NT:NT	22	14	184	22

NA: not available.

chemoprophylaxis to close contacts for prevention of secondary IMD cases.

Internationally, policies vary but vaccination is generally restricted to high-risk groups or as part of outbreak management. Exceptions include the United States where all 11–18 year olds are offered the conjugate quadrivalent MenACWY vaccine [9]. In August 2015, and in response to rapid expansion of the endemic hyper-virulent MenW cc11 strain, a MenACWY catch-up programme was introduced in the UK for young people aged 14–18 and those aged less than 25 years and attending university for the first time [10]. In England and Wales, vaccination against MenW is also recommended for travellers to MenW endemic countries, for at-risk individuals with conditions such as asplenia, hyposplenia and complement deficiency, and also for close contacts of patients with IMD caused by this serogroup [1].

It is important that clinicians are aware of the changing IMD epidemiology and the unique characteristics of MenW that differentiate this capsular group from the classical presentations associated with MenB or MenC. IMD generally can be difficult to diagnose because symptoms are often similar to a viral illness, especially in the early stages of illness (e.g. fever, headache, runny nose). Classical symptoms associated with IMD such as the non-blanching rash, neck stiffness and impaired consciousness tend to present later, by which time the patient is already seriously ill and more likely to have an adverse outcome [11]. In our cohort, information on clinical presentation was derived from information recorded when the patient arrived at the hospital emergency department. Notably, more than half of the patients did not have any of the classical symptoms of meningococcal disease. This is consistent with published reports confirming that patients with MenW are more likely to develop non-meningeal manifestations such as arthritis, pericarditis and pneumonia [12-14]. In France, analysis of all cases of meningococcal disease between 1999 and 2002 found that MenW was significantly more likely than other capsular groups to be associated with meningococcal arthritis and meningococcal pneumonia [12]. Two patients in our cohort presented with cellulitis, which is a rare manifestation of IMD, previously only reported in individual case reports or small case studies and more often associated with MenY or MenB [15,16]. IMD presentation with common gastrointestinal symptoms such as diarrhoea and vomiting has also been reported in the literature. In one study, for example, 24 patients were erroneously hospitalised for gastrointestinal symptoms (with two having appendectomy) and were subsequently found to have meningococcal disease [12]. The age distribution of MenW cases is also different to the more common MenB or MenC cases. In our cohort, for example, half the cases were diagnosed in older adults, who would generally not be considered to have IMD, especially if they develop atypical clinical manifestations such as pneumonia or septic arthritis.

An important consequence of the unusual clinical presentation of MenW disease is that a diagnosis of IMD is often not considered until *N. meningitidis* is isolated from sterile-site cultures (blood, cerebrospinal fluid (CSF), joint), which on average takes 24–72 hours. Confirmation of capsular group can take longer because this requires isolate submission to the national reference laboratory. While PCR confirmation of IMD and capsular group may be quicker, it requires the clinician to consider IMD in the differential diagnosis. In

England and Wales, local hospital laboratories do not offer PCR-testing for meningococcal disease. Therefore, the sterile-site samples (blood/CSF/joint) have to be submitted to the national reference laboratory (MRU), which offers a free national PCR-testing service. This is less likely to occur if patients have non-specific and/or atypical clinical presentations, which is more likely to occur with MenW. The submission of all meningococcal isolates to the MRU, irrespective of PCR investigations, is essential for continued national surveillance, particularly in light of the two new meningococcal immunisation programmes introduced in 2015 [17].

The analysis presented here is limited by the small number of cases, but we have identified a clear increase in MenW cases locally, which is consistent with national trends. The recent emergence of a single strain responsible for most of the recent MenW cases and the lack of a travel history in most patients suggest that this strain is endemic and could lead to further increases in MenW cases. Moreover, although HPZone records limited clinical data, we found that a considerable proportion of cases did not have the classical symptoms of IMD and, as a result, diagnosis and treatment (as well as public health notification and antibiotic chemoprophylaxis for close contacts) may be delayed. Clinicians should, therefore, be aware of the national increase in MenW cases, the atypical clinical presentations associated with MenW disease and the importance of early notification of confirmed cases to local health protection teams to ensure timely chemoprophylaxis and vaccination are offered to close contacts.

Conflict of interest

None declared.

Authors' contributions

Jane Bethea designed the study, completed data collection and analysis and wrote the first full draft of the article. Shamez Ladhani provided advice on overall approach to the study and made a significant contribution to the writing of the final manuscript. Sophia Makki provided advice on the initial development of the study, on data collection and interpretation and commented on the final draft of the article. Steve Gray provided national data for validation purposes, data on method of identification and type/subtype and also commented on all drafts of the article. Vanessa MacGregor provided advice on interpretation of the data, data collection and commented on the final draft of the article.

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Research article

Preparing to introduce the varicella vaccine into the Italian immunisation programme: varicella-related hospitalisations in Tuscany, 2004–2012

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A universal immunisation programme against varicella in the form of the measles-mumps-rubella-varicella (MMRV) vaccine for toddlers aged 13–15 months was introduced in Tuscany in July 2008. An assessment of the impact of this programme on varicella-related hospitalisations 4 years after its introduction could further support its adoption at a national level. The hospitalisation data were analysed in two periods: pre-vaccination (2004–2007) and vaccination period (2009–2012). The high coverage of the vaccines (84% in 2012) resulted in a significant decline in notifications, from 33,114 (2004–2007) to 13,184 cases (2009–2012), and also of hospitalisations, from 584 (pre-vaccination period) to 325 (vaccination period). The hospitalisation rate was 4.1 per 100,000 (95% confidence intervals (CI): 3.4–4.7) before the introduction of vaccination, which dropped to 2.2 per 100,000 (95% CI: 1.7–2.7) in the vaccination period (hospitalisation risk ratios: 0.54; 95% CI: 0.472–0.619). The reduction was most significant in the youngest age groups. The introduction of universal vaccination has already led to a significant decline in hospitalisations due to varicella after just 4 years of implementation. Hospitalisation rates fell noticeably among younger individuals involved in the vaccination programme. The decrease in hospitalisation rate in the older age groups suggests a possible indirect protection.

Introduction

Varicella (chickenpox) is the primary manifestation of infection with the varicella zoster virus (VZV) and is a widespread and highly contagious infectious disease. Today, varicella is recognised as the most common exanthematic disease in children in Italy, with an estimated number of 500,000 new cases each year [1,2]. Seroprevalence studies confirm that VZV infection is still predominantly a paediatric infection in Italy, and that there are no substantial differences between blood samples collected in 1996–1997 and those collected in 2003–2004 [2,3].

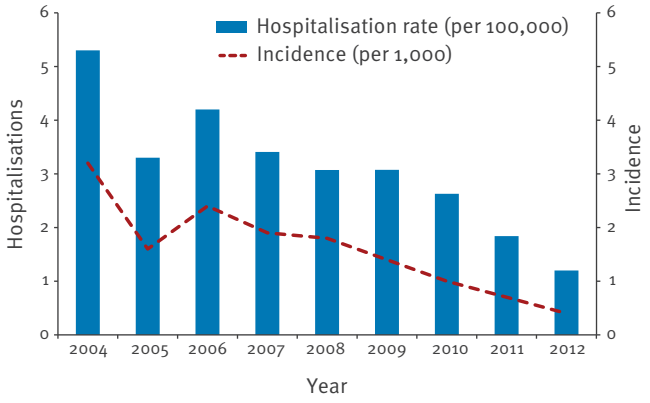
An effective and safe monovalent varicella vaccine has been available in Italy since 1995. However, varicella immunisation has not been widely administered in Italy in the previous decades. In the National Vaccination Plan 2005–2007, the varicella vaccine was recommended only to individuals with a high risk of complications and to susceptible adolescents [4]. However, in order to avoid a shift in the median age of VZV infection towards adulthood, the plan advised introduction of routine childhood varicella vaccination in regions that have been able to achieve and maintain high vaccination coverage against measles, mumps and rubella (MMR) [4]. As a result of these indications, universal immunisation against varicella was not homogeneously implemented at the national level [5,6]. According to an earlier survey of vaccination coverage carried out in Italy by the National Institute of Health (Istituto Superiore di Sanità), the reported childhood vaccination coverage, involving one dose of the varicella vaccine at 24 months of age, was only 17.1% (range: 15.7–18.6%) in Italy in 2008 [7].

In the Italian National Vaccination Plan 2012–2014, (which is the schedule currently being used in 2016) the implementation of universal varicella vaccination at the national level was pushed back until such time when the monitoring and data evaluation of the pilot programmes carried out in some Italian regions (Basilicata, Calabria, the autonomous province of Bolzano, Puglia, Sardinia, Sicily, Tuscany, and Veneto) would be available [8]. Assessment of the epidemiological and economic impact of immunisation against varicella through these regional programmes will enable the rationale for the aforementioned decision to be validated. At the time of publication, the evaluation and related implementation at the national level have not yet been performed.

Tuscany was one of the first Italian regions to introduce and implement a universal vaccination programme

Figure 1

Hospitalisation rate for varicella diseases and incidence of varicella, Tuscany, Italy, 2004–2012



against varicella with a combined MMRV vaccine for children aged 13–15 months (first dose) and 5 to 6 years (second dose), in July 2008 [9]. The impact of this preventive intervention was evaluated 4 years after the implementation.

In particular, the objective of our study is to evaluate the impact of universal varicella vaccination in Tuscany by analysing all varicella-related hospitalisations reported in the period 2004–2012.

Methods

The hospital discharge records related to varicella were analysed in order to evaluate the impact of universal varicella vaccination on the hospitalisation rate in Tuscany. Hospitalisation data for 2004–2012 were collected from the Tuscan regional database. In particular, all hospitalised cases for varicella or its complications, as a primary or secondary discharge diagnosis, with the following ICD-9-CM codes (2002 and 2007) were examined: 052.0 (post-varicella encephalitis), 052.1 (varicella (haemorrhagic) pneumonitis), 052.2 (post-varicella myelitis), 052.7 (varicella with other specified complications), 052.8 (varicella with unspecified complication), and 052.9 (varicella without complication) [10].

For each hospitalised case, the collected information included year of hospitalisation, age at time of discharge, sex, region of origin, local health unit, country of residence, hospital days, primary and up to five secondary diagnoses, and total cost of hospitalisation. The costs of hospitalisation correspond to the refunding by the region to the hospitals. Costs are calculated according to the diagnosis-related group (DRG), and also include the possible costs due to the additional days of hospitalisation in excess of the specific outlier threshold. Moreover, the resident population was grouped by age, as retrieved by the Italian National Institute of Statistics database, in order to calculate hospitalisation rates [11].

Hospitalisation data were compared with surveillance data obtained from the Tuscan regional database for varicella cases notified in Tuscany in the same period.

In addition, data on varicella vaccine coverage in children aged 24 months in the period 2008–2012 were included in the analysis. Data on vaccination coverage were also collected from the Tuscan regional database.

Varicella-related hospitalisations were analysed as follows: (i) in the entire period 2004–2012, in order to evaluate the trend over time; (ii) in two periods of 4 years each (the pre-vaccination period, 2004–2007; and the period following the vaccine's introduction, 2009–2012). Data from 2008, the transition year between the two periods mentioned above, were excluded from our analysis. The impact of universal varicella vaccination was assessed by calculating the hospitalisation risk ratios (HRRs) with a 95% confidence interval (CI) (Mantel-Haenszel combined test).

Results

Varicella vaccination in Tuscany

Inclusion of the quadrivalent combined MMRV vaccine in the immunisation programme led to rapid achievement of high vaccine coverage against varicella in Tuscany, with a relevant dragging effect. In fact, varicella vaccination with MMRV or monovalent varicella vaccines for children at 24 months of age achieved high coverage soon after implementation: 75.5% in 2010, 82.2% in 2011, and 84.0% in 2012.

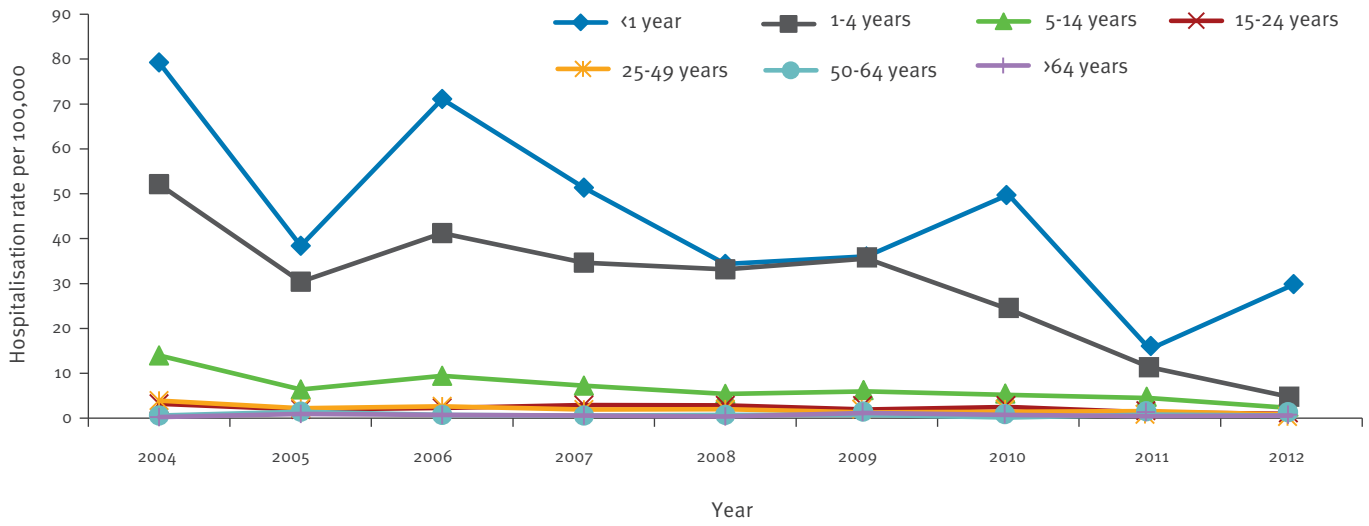
Varicella cases and hospitalisations

In Tuscany, 52,738 varicella cases were notified to the Regional Health Authority in 2004–2012. A dramatic overall reduction in the number of notifications was reported between 2004 (11,445 cases) and 2012 (1,502 cases). The number of reported varicella cases in the vaccination period (13,184 cases) has been reduced by more than half (60.2% reduction) of that in the pre-vaccination period (33,114 cases). The average incidence rate has declined from 2.30 cases (95% CI: 2.25–2.35) to 0.89 cases (95% CI: 0.86–0.92) per 1,000 inhabitants.

For the same period in 2004–2012, a total of 1,022 hospital discharge records related to varicella or its complications were found in Tuscany. The number of hospitalisations due to varicella diseases showed a decreasing trend over the 9 years analysed: 189 hospitalisations for varicella (5.3 hospitalised cases/100,000 inhabitants; 95% CI: 4.5–6.1) were reported in 2004 and 44 in 2012 (1.2/100,000 inhabitants; 95% CI: 0.8–1.6). In other words, there was a substantial reduction in the number of hospitalisations (by 77%) between 2004 and 2012. The incidence of notified varicella cases and the rate of hospitalised cases for varicella diseases showed the same trend in the period 2004–2012 in Tuscany (Figure 1).

Figure 2

Hospitalisation rates for varicella, by age group, Tuscany, Italy, 2004–2012



In the pre-vaccination period (2004–2007), there were 584 hospitalised cases related to varicella or its complications in Tuscany, while in the vaccination period (2009–2012) there were 325. A total reduction of 44% (259 fewer cases) in the number of hospitalisations due to varicella was observed between 2009 and 2012. In particular, the average hospitalisation rate for varicella diseases was 4.1 per 100,000 (95% CI: 3.4–4.7) in the period before the introduction of varicella vaccination in Tuscany. This dropped to 2.2 per 100,000 (95% CI: 1.7–2.7) in the vaccination period (HRR: 0.54; 95% CI: 0.47–0.62).

Hospitalisations for varicella diseases analysed by age at time of discharge

In the period 2004–2012, the hospitalisation rates related to varicella or its complications showed a downward trend in almost all age groups, especially for children under 14 years of age. Children aged between 1 and 4 years, who had historically been affected by varicella diseases and were enrolled in the current universal programme of varicella vaccination in Tuscany, appeared to be the age group that received the greatest benefit from vaccination (a net reduction in hospitalised cases by 90% in the 9-year period, from 60 cases in 2004 to six cases in 2012, corresponding to a hospitalisation rate of 52.1 (95% CI: 38.9–65.3) and 4.6 (95% CI: 0.9–8.4) per 100,000 inhabitants respectively) (Figure 2).

On comparing the pre-vaccination and vaccination period data, the most relevant reduction (>20%) in the rate of hospitalisation due to varicella diseases was again reported in children below 5 years of age (Table).

Hospital discharge records for varicella diseases analysed by discharge code

The hospital discharge records for primary and secondary diagnoses of varicella or its complications in the study period (2004–2012) were analysed. Among the 1,022 hospitalised cases in Tuscany, a total of 1,041 varicella-related codes (ICD-9-CM) were reported. Of these, 19 hospitalised cases had more than one code identifying varicella diseases in the discharge diagnosis. The most frequently reported discharge code was varicella without complication (052.9). In the period 2004–2012, there were no hospitalisations for post-varicella myelitis (ICD-9-CM 2007 code 052.2).

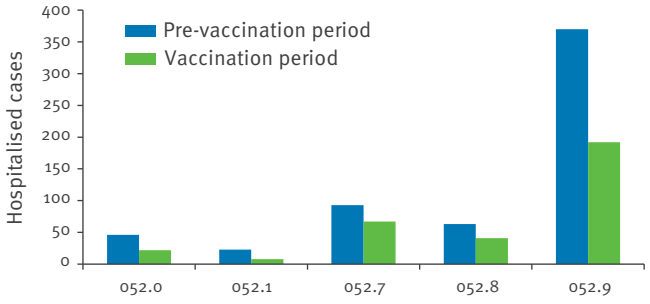
In the switch from the pre-vaccination years (2004–2007) to the vaccination period (2009–2012), a net reduction in hospitalised cases was evident for all discharge codes related to varicella. The greatest reduction in the number of hospitalised cases (178 cases) between the two periods (2004–2007 vs 2009–2012) pertained to the discharge diagnosis of varicella without complications (052.9) (Figure 3). In fact, hospitalisations for this diagnosis decreased by 10.5% in individuals aged 1–14 years who were involved in the vaccination programme.

Total days of hospitalisation for varicella diseases

In the period 2004–2012, a total of 1,022 patients hospitalised for varicella or its complications in Tuscany remained in the hospital for 6,112 days (an average of six hospital days per patient). The total number of hospital days showed a clear downward trend in the 9 years analysed. In particular, while in 2004 patients were admitted for 960 days in total, in 2012 the total number of hospital days had decreased to 378 days, with an overall reduction of 61% between the first and the last year analysed in the study. In the pre-vaccination period (2004–2007), the total number of days hospitalised was 3,388; this number decreased to 2,021 in the vaccination period (2009–2012), showing a reduction of 40% (1,367 days fewer). The average number of hospital days per patient ranged from 5.8 to 6.6 in the pre-vaccination and vaccination periods.

Figure 3

Number of hospitalised cases for varicella diseases, by diagnosis code[a], Tuscany, Italy, in the pre-vaccination (2004–2007) and vaccination periods (2009–2012)



[a] Reference [10].

International classification of diseases, ninth revision, clinical modification (ICD-9-CM) codes: 052.0 (post-varicella encephalitis), 052.1 (varicella (haemorrhagic) pneumonitis), 052.2 (postvaricella myelitis), 052.7 (varicella with other specified complications), 052.8 (varicella with unspecified complication), and 052.9 (varicella without complication)

Figure 4

Total cost of hospitalisation for varicella diseases, by age group, Tuscany, Italy, pre-vaccination (2004–2007) and vaccination periods (2009–2012)

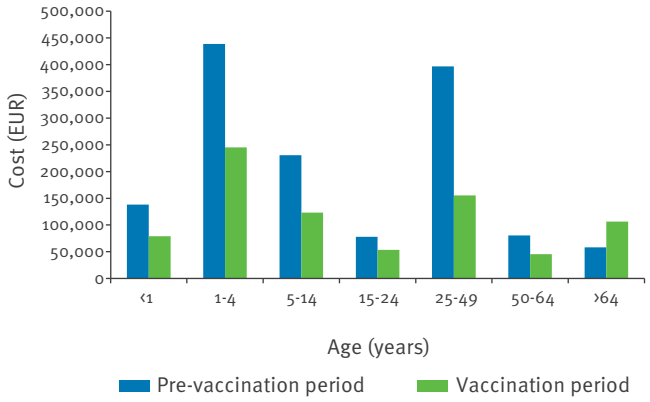
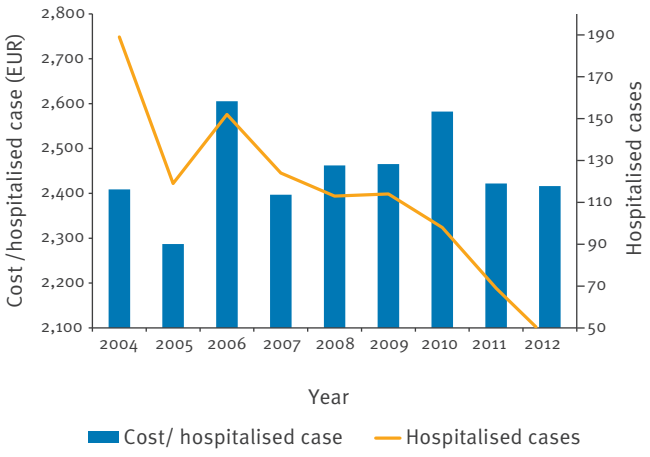


Figure 5

Cost per hospitalised case for varicella diseases, Tuscany, Italy, 2004–2012



Cost of hospitalisations due to varicella diseases

In the period 2004–2012, the cost of the 1,022 hospitalisations for varicella or its complications in Tuscany amounted to EUR 2,506,422 (EUR 2,452 per patient on average). The total annual cost of hospitalisation has shown a decreasing trend in the 9 years analysed, similar to the trend in hospitalised cases. In particular, while 189 hospitalisations for varicella occurred in 2004, incurring a cost of EUR 455,267, in the final year of the study 44 patients were hospitalised for a total cost of EUR 106,311, an overall cost reduction of 77%. In the pre-vaccination period (2004–2007) the total cost of hospitalisation due to varicella was EUR 1,420,657 (EUR 355,164/year). In the vaccination period (2009–2012), the cost decreased to EUR 807,535 (EUR 201,884/year). Therefore, hospital costs declined by 43%, creating total savings of EUR 613,121, or EUR 153,280/year.

In the switch from the pre-vaccination period (2004–2007) to the vaccination period (2009–2012), a reduction in the total cost of hospitalisations for varicella diseases was reported in all age groups in Tuscany (Figure 4). However, the cost of hospitalisation increased among individuals over 64 years of age, though the number of hospitalised patients in this age group had not changed much from the pre-vaccination period (21 cases) to the vaccination period (25 cases). This slight increase in costs is mainly attributable to the specific case of an 80-year old patient whose primary diagnosis was pulmonary insufficiency, with secondary diagnoses including generalised convulsive epilepsy, cerebrovascular disease, a kidney disorder, and post-varicella encephalitis. This patient's hospital costs amounted to EUR 34,724. The greatest reduction of hospitalisation costs in absolute number (EUR 241,267) concerns the age group of 25–49 years (data not shown).

In the period 2004–2012, although the number of hospitalised cases and the total annual cost of hospitalisation declined to a great extent, the cost per hospitalised case seemed to show a slight increasing trend over the 9 years analysed (Figure 5). In the pre-vaccination period (2004–2007), the average cost of hospitalised cases was EUR 2,433 per patient, while in the period of varicella vaccination (2009–2012), this cost increased by 2% to EUR 2,485, with an increment of EUR 52 in the average hospitalisation cost of each patient.

Discussion and conclusions

The routine immunisation of toddlers against varicella has not yet been widely implemented in Europe. Assessment of the epidemiology and disease burden of varicella in each country and evidence for the effectiveness of varicella vaccination could provide support for decision on implementation of routine childhood programmes [12]. Generally, only a small percentage of people infected with varicella diseases are hospitalised in Italy. However, the period 2000–2003 saw an

Table

Hospitalisations for varicella diseases, by age, and hospitalisation risk ratios with 95% CI, Tuscany, Italy, pre-vaccination (2004–2007) and vaccination periods (2009–2012)

Age group	Pre-vaccination period 2004–2007		Vaccination period 2009–2012		HRR	95% CI
	Hospitalisations	Average rate per 100,000	Hospitalisations	Average rate per 100,000		
‹1 year	73	59.6	42	32.7	0.55	0.38–0.80
1–4 years	189	39.5	99	18.9	0.48	0.38–0.61
5–14 years	105	9.2	55	4.5	0.48	0.35–0.67
15–24 years	32	2.6	21	1.7	0.64	0.37–1.19
25–49 years	141	2.7	67	1.3	0.47	0.35–0.63
50–64 years	23	0.8	16	0.5	0.67	0.35–1.26
›64 years	21	0.6	25	0.7	1.14	0.64–2.04
Overall	584	4.1	325	2.2	0.54	0.47–0.62

CI: confidence interval; HRR: hospitalisation risk ratio.

annual average of 1,575 hospitalisations where varicella was the primary diagnosis (1,521 hospitalisations and 54 day-hospital admissions) with a mean hospital stay of 5.3 days per person [2]. Approximately one-third of these cases occurred in people older than 14 years and could have been prevented through a programme of universal vaccination, including an active offer of varicella vaccine to adolescents and adults at high risk without any anamnestic history of varicella diseases [2].

The results of our study show that the introduction of a universal varicella vaccination with MMRV vaccine in Tuscany for all newborns has already resulted in a significant reduction in varicella notifications as well as in the number of hospitalisations due to varicella and related costs, within the first 4 years of implementation. In particular, the current analysis highlights that the number of hospitalised cases has been greatly reduced, especially among younger individuals who were involved in the vaccination programme. However, a positive impact of vaccination on the hospitalisation rate is also evident in other age groups, suggesting a herd immunity effect. Our data on significant reduction of hospitalisation due to varicella in adults are compatible with the results published in Canada [13] and the United States (US) [14] but contradict the hospitalisation data in Germany, where results do not demonstrate herd protection against varicella with 87% vaccination coverage [15]. In addition, the absence of a shift of infection time to the older age groups could be explained by the high vaccination coverage among children. Comparing hospitalisation for uncomplicated varicella (the most frequent discharge diagnosis) in the pre-vaccination period (2004–2007) and in the vaccination period (2009–2012), a clear reduction is evident, thus suggesting that the slight increase in the cost per hospitalised patient might be due to a higher chance that only the most severe cases were hospitalised during the vaccination period.

Limitations of the study are mainly due to the fact that our data on hospitalisations are based on administrative data. A greater appropriateness of hospital admissions during the examined period cannot be excluded as an additional factor in the reduction of hospitalisation rate. Another possible limitation is that varicella may have been coded as zoster or vice versa, even though we included only hospitalisation related to varicella in the analysis. Therefore, the magnitude of the decline implies a marked positive effect of the rapid increase in the vaccination coverage, achieved due to the availability of the quadrivalent MMRV vaccine.

Our study confirms the remarkably favourable clinical impact on notified cases and varicella-related hospitalisations reported in two other Italian regions (Sicily and Veneto), a few years after the implementation of the immunisation programme. Sicily was the first Italian region to offer a universal active vaccination against varicella free of charge to infants at 15 months of age and to all susceptible adolescents at 12 years of age, in January 2003 [16,17].

The Veneto region adopted active and free universal varicella vaccination in January 2005 for children aged 14 months (offering a second dose at the age of 6 years), and a catch-up programme for adolescents aged 12 years. The rapid achievement of high (but not optimal) coverage of vaccination against varicella (78.6% in the 2008 birth cohort) resulted in a net decrement (halving) of the varicella incidence, both in the 0–14 year age group and in the general population, within 4 years of the introduction of the varicella vaccination programme. Furthermore, in this region, hospitalisation due to varicella infection also significantly decreased within 1.5 years of the introduction of the universal varicella vaccination, confirming a significant positive impact of universal varicella vaccination in this Italian region [18-20].
In addition, the reduction in varicella cases and number of related hospitalisations highlighted in Sicily, Veneto, Tuscany, and recently in other Italian regions [21,22],

after the introduction of the universal varicella immunisation programme, had already been observed in other countries. In the US, universal varicella vaccination with one-dose (1996) and two-dose (2006) schedules for children was demonstrated to have a clear and significant impact on the disease burden of VZV infection within a few years of implementation [23-28]. In our recent data, obtained a few years after the start of the varicella immunisation programme, the number of hospitalisations due to varicella shows a trend similar to the results reported in the US [29-35].

A relevant reduction of VZV infections and disease complications has also been observed in Germany, which was the first European country to implement a routine universal vaccination programme against varicella in 2004 [36-38].

From an economic point of view, the universal vaccination of children in Tuscany led to savings amounting to EUR 613,121 over 4 years (EUR 153,280/year), considering only the potential costs of hospitalisations that were prevented by immunisation. In particular, a reduction in hospitalisation costs is evident in all age groups except for individuals over 64 years, where the high cost during the vaccination period can be mainly attributed to the previously described case of a patient aged 80 years who had other medical conditions and incurred hospitalisation costs amounting to EUR 34,724. However, the reported savings could be even more substantial if it is assumed that the real expense is 30–40% higher than the figure calculated through the DRG value, as shown by Azzari et al. [39]. In addition, in our study, we analysed only the cost of hospitalisations due to VZV infection: savings following vaccination would have greatly increased if we also included the other clinical costs and indirect costs due to productivity loss. Therefore, the data we collected in a real-setting scenario seem to confirm the favourable economic contribution of varicella immunisation in Italy, as previously reported in three Italian model-based evaluations [40-42]. Particularly in the mathematic simulation performed by Bonanni et al., a varicella vaccination programme with two doses for toddlers was predicted to lead to reduction by more than 80% in VZV infection and hospitalisation rate in a 30-year time horizon [42]. The results of our study show a 44% reduction in the rate of hospitalisation due to varicella within 4 years of the introduction of varicella vaccination, confirming the model's mathematical forecast.

In conclusion, our experience clearly supports the intention of the Italian health authorities to introduce a national universal routine vaccination against varicella. The use of the quadrivalent MMRV vaccine is a key element to achieving high vaccination coverage in a short period of time, thus speeding up the impact on disease incidence and related hospitalisations. The Italian regional vaccination programmes against varicella may also prove to be a useful example for other countries where the introduction of routine toddler vaccination is being considered.

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Conflict of interest

All authors received fees from vaccine producers (GSK, SPMSD, Pfizer, Novartis) for taking part to advisory boards or expert meetings, and grants for acting as investigators (PB) in epidemiological studies co-funded by vaccine producers (Pfizer, GSK).

Authors' contributions

All the authors contributed equally in the design, collection and analysis of data.

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