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Insidious reintroduction of wild poliovirus into Israel, 2013

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Israel was certified as polio-free country in June 2002, along with the rest of the World Health Organization European Region. Some 11 years later, wild-type polio virus 1 (WPV1) was isolated initially from routine sewage samples collected between 7 and 13 April 2013 in two cities in the Southern district. WPV1-specific analysis of samples indicated WPV1 introduction into that area in early February 2013. National supplementary immunisation with oral polio vaccine has been ongoing since August 2013.

Detection of wild poliovirus in Israel

Wild poliovirus type 1 (WPV1) was isolated from routine samples collected during epidemiological week 15 (7 to 13 April 2013) in sewage treatment facilities in Beer Sheva and Rahat, two cities in Israel's Southern district. Beer Sheva, the regional commercial centre, has a population of about 200,000. It is situated 20 km south of Rahat and there is considerable population movement and commerce between the two cities.

Background

Poliomyelitis (polio) has been a notifiable disease in Israel since 1951. WPV1 predominated during the large, pre-vaccine national epidemics during 1949 to 1956 [1,2] with a peak incidence of 129.4 cases per 100,000 of the general (mostly Jewish) population in 1950 [1]. Substantial control of polio was achieved by the mid-1960s (0.1 per 100,000 in 1965) through universal childhood vaccination with trivalent oral polio vaccine (OPV), followed by low-level activity throughout the 1970s (0.2-0.9 per 100,000), particularly in the Jewish population (0.1 per 100,000 Jewish population). Annual campaigns with type 1 monovalent OPV further reduced polio incidence also among the non-Jewish population in the 1980s (0.1 to 0.3 per 100,000 non-Jewish population) [1].

Israel has not had a polio case since an outbreak in 1988, in which 15 poliovirus-infected people had paralytic polio, mostly in the Hadera sub-district in the northern part of the country [1,3], following importation of WPV1 from northern Egypt the year before the outbreak [1,4]. Most of the 1988 outbreak cases had received OPV in the past. A mass trivalent OPV vaccination campaign ended the outbreak [3].

In 1990, a routine national vaccine programme – combining three inactivated polio vaccine (IPV) doses with three trivalent OPV doses – was implemented. This schedule continued until 2005, when OPV was discontinued three years after the polio-free status certification by the World Health Organization (WHO), in June

TABLE 1*

Historical and current routine childhood vaccine schedules against poliomyelitis, Israel, 1957–2013

Age	1957–1960	1961–1989 ^a	1990–2004	2005–present
2 months	–	OPV	IPV	IPV
4 months	IPV	OPV	OPV + IPV	IPV
6 months	IPV	OPV	OPV	IPV
12 months	IPV	OPV	OPV + IPV	IPV
6 years	–	–	OPV	–
7 years	–	–	–	IPV
13 years	–	–	OPV	–

IPV: inactivated polio vaccine; OPV: oral polio vaccine (trivalent).

^a Between 1982 and 1988, two of the 15 sub-districts in the country used IPV at the age of 2, 3.5 and 10 months.

2002 [1]. The IPV-only schedule since 2005 prescribes three IPV doses by the age of six months with two additional booster doses at 12 months and at seven years (second elementary grade) (Table 1). The 2012 national IPV3 coverage was 95% [5].

Israel has maintained a routine environmental polio surveillance programme since 1988, through monthly sampling of eight to 10 sewage treatment facilities in largely populated areas or areas considered sentinels of risk for importation of WPV, such as Rahat, a major Bedouin city, which was the initial importation locus of the 1988 WPV1 outbreak [4]. Additionally, since 1996, notification of cases with acute flaccid paralysis (AFP) up to the age of 15 years has been mandated by law and active AFP surveillance has been implemented. Since then, the level of reporting fluctuated, sometimes below the WHO threshold (1 case of AFP per 100,000 population <15 years of age). As a consequence, the structure of the active AFP surveillance system was reorganised several times in the past years, and since January 2013, the national Division of Epidemiology contact directly, on a weekly basis, each representative of the relevant clinical care units in the country, in order to obtain the complete information on any suspected AFP cases, and assuring the required clinical sampling for the necessary laboratory tests.

Here we describe the epidemiology of the reintroduction of WPV1 into Israel in 2013, a potential public health emergency at the national level and public health threat at the global level.

Epidemiological investigation

Environmental surveillance

As part of the regular environmental surveillance, sewage samples are processed at the Central Virology Laboratory in Tel Hashomer. Poliovirus is isolated from the samples by infecting recombinant mouse cell line L20B according to WHO guidelines [6,7]. Individual plaques propagated in tube cultures are then analysed by real-time polymerase chain reaction (PCR) using United States (US) Centers for Disease Control and Prevention (CDC) analytical kits designed for identification of polioviruses and differentiation between vaccine and wild types [8], followed by characterisation by genome sequencing [9].

The samples collected in the sewage treatment facilities of Rahat and Beer Sheva are part of this routine environmental surveillance. At the end of May 2013, the Central Virology Laboratory confirmed that WPV1 had been detected in two sewage samples taken in the Rahat and Beer Sheva facilities in epidemiological week 15 (7-13 April 2013).

A WPV1-specific analysis of samples from early 2013 indicated WPV1 introduction into Beer Sheva in February 2013 (week 6, 3-9 February) and into Rahat in March 2013 (week 11, 10-16 March). The isolates from

Rahat and Beer Sheva were identified as non-Sabin poliovirus type 1 belonging to the SOAS (South Asia) lineage of WPV1, which has been circulating in Pakistan in recent years, and which was also isolated from sewage samples in the Cairo region, Egypt, in December 2012 [10].

Intensified environmental surveillance

Subsequently, environmental surveillance was extended and intensified to cover more sewage sampling sites nationally. As of 1 September 2013, WPV1 has been detected in 87 of 220 samples tested that were obtained from 79 sewage sampling sites in Israel and collected after 3 February 2013 (Figure). In the southern district, most of the treatment facilities with samples that have been continuously WPV1 positive to date have been in areas inhabited by Bedouin communities. WPV1 was also detected in several sewage sampling sites in central Israel, mostly around Arab or mixed Jewish–Arab communities, indicating country-wide transmission.

Public health response from end May to 5 August 2013

The following instant actions were carried out, following the detection of WPV1 in Israel.

- In Rahat and the surrounding area, where substantial and continuous WPV1 circulation has been detected in samples collected since the first quarter of 2013, an IPV catch-up vaccination campaign was initiated in order to maximise the routine childhood IPV coverage and to administer a booster IPV to all adults who had no evidence of prior booster vaccination in adulthood (i.e. before travel to polio endemic countries), with special outreach to sewage facility workers and migrant communities whose members had migrated through Egypt since 2007 [11].
- A national hygiene campaign was initiated to raise public awareness of wild poliovirus circulation and hand-washing and personal hygiene and in preparation for a widespread OPV supplementary immunisation activity.
- In order to maximise OPV coverage, professional information concerning poliovirus and vaccination was made available to physicians throughout the country as well as to the public, using diverse communication channels – traditional media, social media, and the Internet [12].

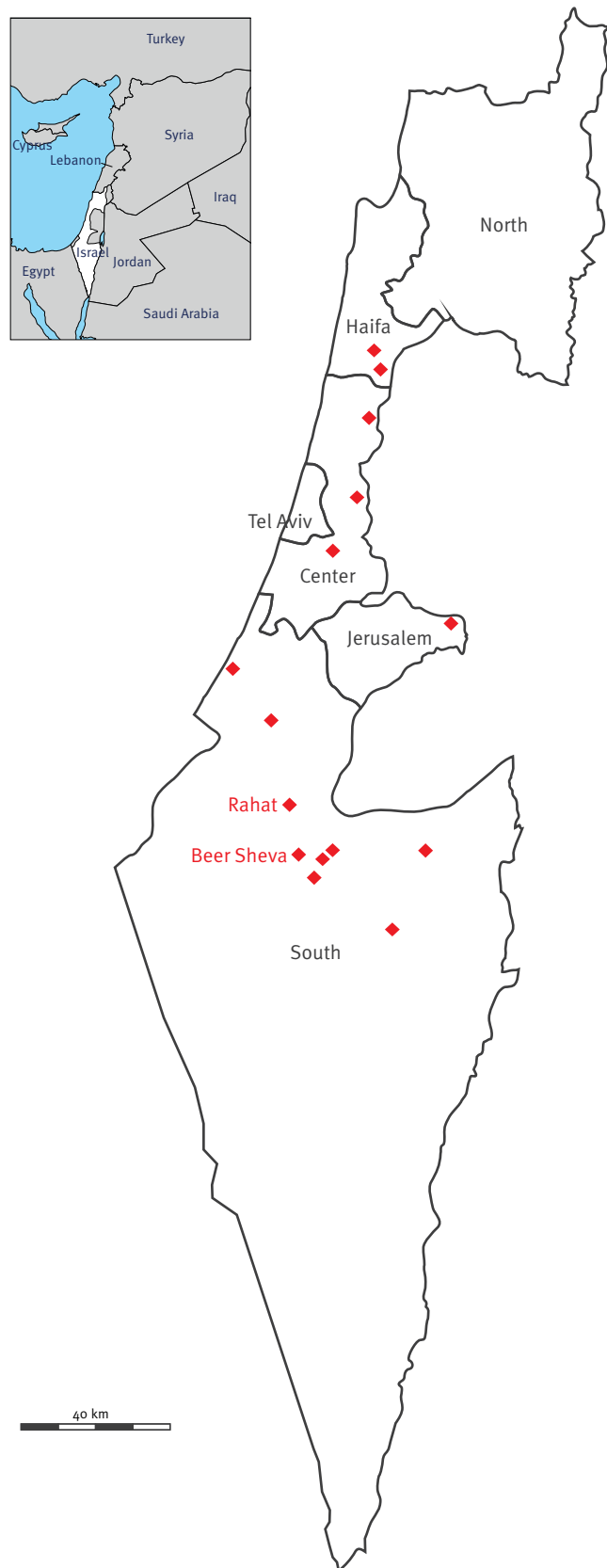
Enhanced surveillance of acute flaccid paralysis

After the isolation of WPV1 in sewage, surveillance for AFP was expanded in June 2013, to include all age groups.

From 1 January 2013 to 1 September 2013, 45 cases of AFP had been actively detected, of which, 17 were children <15 years of age. Three of these children were

FIGURE

Locations of sewage samples positive for wild poliovirus type 1 identified through environmental surveillance, Israel, by district, 3 February–12 September 2013



defined as ‘hot’ cases (i.e. lacking a complete series of routine childhood IPV immunisation due to young age or parental objection) [13]. The estimated annual AFP incidence in 2013, based on cases detected so far, is 1.15 cases per 100,000 population <15 years of age. All 45 AFP cases tested negative for WPV1.

No case of paralytic polio has been detected to date in Israel.

Start of aseptic meningitis surveillance

National aseptic meningitis surveillance was also initiated in June 2013. As of 28 August 2013, a total of 156 cases of aseptic meningitis were reported nationally: none was positive for poliovirus; 65 cases (42%) were positive for other non-polio enteroviruses.

Ongoing national supplementary immunisation activity

A supplementary immunisation activity using bivalent OPV [14,15] was initiated in the Southern district on 5 August 2013 and has been expanded nationally since 18 August, with the objective of rapidly interrupting WPV1 transmission across the country, particularly in children previously vaccinated according to the IPV-only programme, by inducing effective intestinal immunity [16,17].

All children born after 1 January 2004, who have received at least one dose of IPV in the past, were considered vaccine candidates. This approach is in accordance with that used in the routine national immunisation schedule during 1990 to 2004, which has a formidable international safety profile [18]. Vaccine candidates who have immunodeficiency conditions or those living with immunocompromised household contacts are not vaccinated with bivalent OPV.

As of 15 September 2013, approximately 750,000 of about 1,200,000 eligible bivalent OPV candidates (63%, inter-district range: 45–83%) were vaccinated nationwide (Table 2).

The Israeli response to the finding of WPV1 has been fully coordinated with leading local epidemiology, infection disease and paediatric experts, as well as WHO and the US CDC. A joint WHO/CDC mission visited Israel in June 2013 and its experts have been consulted at every stage of the outbreak response.

Discussion

The last widespread circulation of wild poliovirus in Israel was 25 years ago, resulting in a national outbreak with cases of permanent paralytic polio [3]. The major difference between the 1988 outbreak in Israel and other outbreaks of wild poliovirus infection in recent years in other developed countries [19,20] that used an IPV-only routine vaccination schedule, is the early detection of silent virus circulation through an existing early warning system, involving national environmental

TABLE 2

Bivalent oral polio vaccine coverage^{a,b}, by district, since the start of the supplementary immunisation activity on 5 August 2013, Israel

District	Vaccine coverage (%)
Northern	83
Southern ^c	79
Haifa	69
Ashkelon	63
Central	54
Tel Aviv	49
Jerusalem	45
Total	63

^a Among about 1,200,000 eligible vaccine candidates, born after 1 January 2004.

^b Data as of 15 September.

^c In the Bedouin population of the Southern district communities, the bivalent oral polio vaccine coverage surpassed 80%.

surveillance. This early detection allowed for further investigation and planning of a national response well in time.

An apparent single event of long-distance WPV1 importation from Pakistan, where the SOAS genotype has circulated in 2012, to Egypt, possibly by an asymptomatic carrier or carriers, and the further importation to Israel – a journey of thousands of kilometres – has resulted in nearly nationwide WPV1 spread. This widespread WPV1 circulation might have been facilitated by the substantial non-OPV immunised cohort of children, who were born after the withdrawal of routine OPV doses from the national immunisation programme in 2005.

Notably, the highly probable chain of events that lead to the re-introduction of WPV1 into Israel in 2013 is not the first of its kind. Other possible long-distance point importations into decades-long polio-free countries were identified, in Finland and the Netherlands, following the paralytic polio outbreaks of 1986 and 1992, respectively [19-22]. This is in contrast to the endemic, Middle-Eastern origin of the 1988 outbreak due to WPV1 in Israel [1,3,4].

The detection of WPV1 in 2013 in Israel should therefore alert polio-free countries and global health organisations, which could confront a similar situation, given the increased mobility of people and populations, as long as wild poliovirus continues to be endemic in several parts of the world. Furthermore, our findings are also relevant to the global debate related to polio preventive measures such as routine national environmental surveillance and reinstatement of combined IPV and

OPV routine immunisation schedule, even in polio-free countries with high IPV coverage [23].

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Conflict of interest

None declared.

Authors' contributions

Emilia Anis, Eran Kopel, Shepherd R. Singer and Itamar Grotto wrote the first draft of the manuscript. All authors revised and approved the final version of the manuscript.

* Authors' correction

In the last row of Table 1, '16 years' was corrected to '13 years'. This correction was made on 18 November 2013 at the request of the authors.

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Stability of Middle East respiratory syndrome coronavirus (MERS-CoV) under different environmental conditions

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The stability of Middle East respiratory syndrome coronavirus (MERS-CoV) was determined at 20°C – 40% relative humidity (RH); 30°C – 30% RH and 30°C – 80% RH. MERS-CoV was more stable at low temperature/low humidity conditions and could still be recovered after 48 hours. During aerosolisation of MERS-CoV, no decrease in stability was observed at 20°C – 40% RH. These data suggest the potential of MERS-CoV to be transmitted via contact or fomite transmission due to prolonged environmental presence.

In 2012, a novel coronavirus termed Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the Middle East [1]. Limited human-to-human transmission of MERS-CoV has been observed [2-5] and currently no data are available on the modes of transmission. The occurrence of MERS-CoV as a respiratory pathogen and the high viral loads detected in samples from the lower respiratory tract of infected patients [6,7] suggests that MERS-CoV will be predominantly shed during coughing and via exudates from the lower respiratory tract. For influenza A virus it is shown that transmission is linked to the viability of the virus under different environmental conditions, such as temperature and humidity with a cool dry environment being the most favourable for transmission and either warm or humid conditions being unfavourable [8]. In this study, the stability of MERS-CoV (isolate HCoV-EMC/2012) was evaluated under three different environmental conditions: high temperature and low humidity, 30°C – 30% relative humidity (RH); high temperature and high humidity, 30°C – 80% RH and low temperature and low humidity, 20°C – 40% RH, to reflect a wide range of environmental conditions including an indoor environment (20°C – 40% RH). The stability of MERS-CoV under the three tested environmental conditions was respectively compared with that of influenza A virus A/Mexico/4108/2009 (H1N1) originating from a human isolate obtained during the influenza A(H1N1) pdm09 pandemic in 2009 [9]. The stability of the two viruses in aerosols at 20°C with 40% or 70% RH was also assessed and compared.

Environmental stability

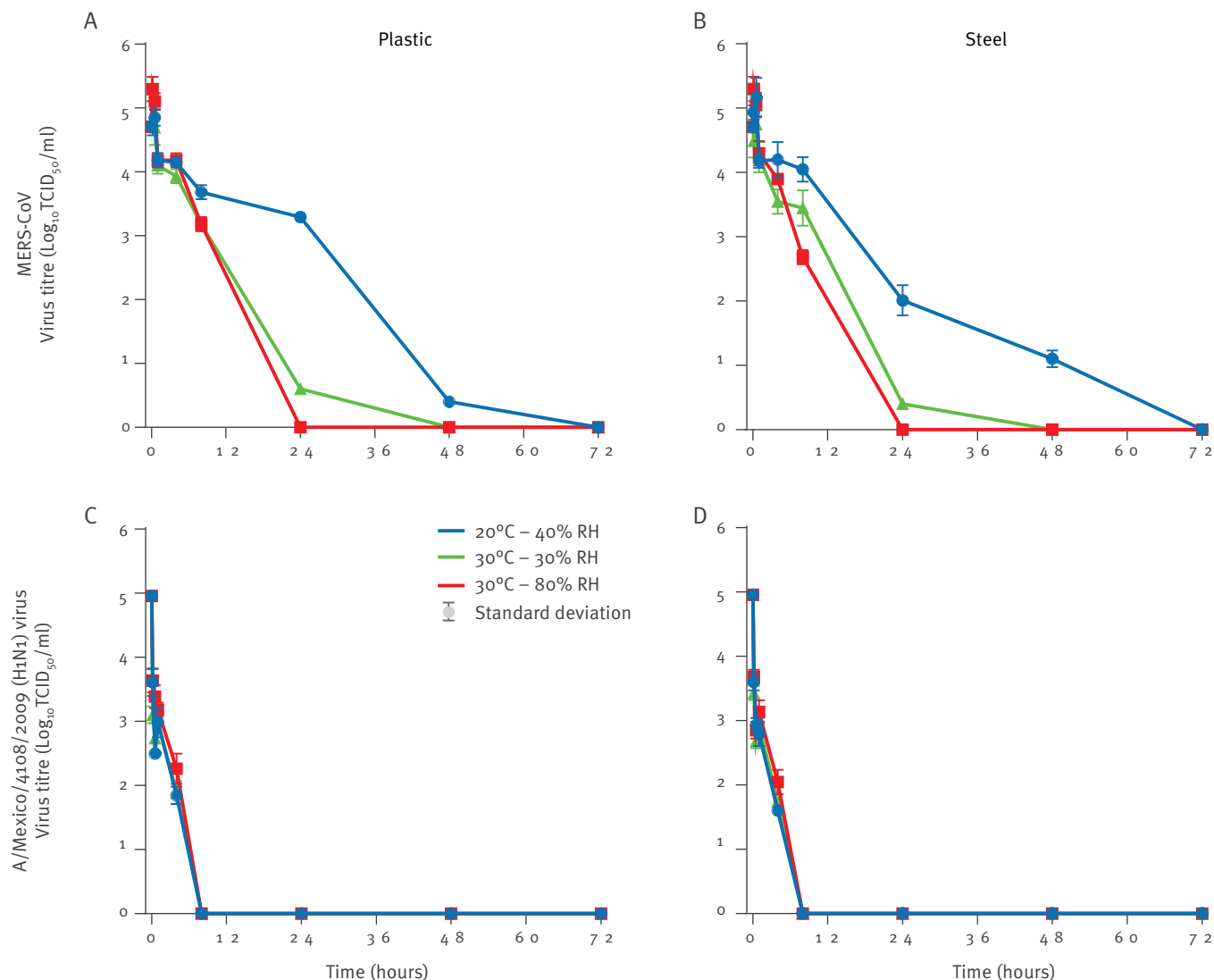
MERS-CoV (isolate HCoV-EMC/2012) and A/Mexico/4108/2009 (H1N1) virus were propagated and titrated by end-point titration on VeroE6 cells (for MERS-CoV) and Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus) as previously described [9,10]. To determine the environmental stability of the two viruses, 100 µl of 10⁶ tissue culture infective dose 50 (TCID₅₀) of MERS-CoV or A/Mexico/4108/2009 (H1N1) virus was spotted in droplets of 5 µl on the surface of steel or plastic washers (McMaster-Carr, USA) and incubated at the desired conditions in an environmental chamber (Caron, USA) for 10 and 30 minutes and 4, 8, 24, 48 and 72 hours. Experiments were conducted in triplicate. For both MERS-CoV and A/Mexico/4108/2009 (H1N1) virus, no differences in stability could be observed between the plastic and steel surface, suggesting the plastic and steel surfaces did not affect the stability differentially (Figure 1, panels A and D). MERS-CoV virus could still be recovered after 48 hours at the 20°C – 40% RH condition, whereas for the other two conditions the virus remained viable for eight (30°C – 80% RH) and 24 hours (30°C – 30% RH) respectively. For A/Mexico/4108/2009 (H1N1) virus, no virus could be recovered after four hours at each environmental condition and no difference between the environmental conditions was observed. The mean half-life values of MERS-CoV varied between 0.441822 and 0.973656 hours (five-parameter logistic model, R and R package drc [11]), but were not found to be significantly different (Table). Due to the rapid decrease in viability of A/Mexico/4108/2009 (H1N1) virus, half-life values could not be calculated.

Aerosol stability

To study their respective aerosol stability, MERS-CoV and A/Mexico/4108/2009 (H1N1) virus were aerosolised at 20°C with 40% or 70% RH (Figure 2). Aerosol experiments were performed using the AeroMP (Biaera Technologies, USA) aerosol management platform [12]. 10⁶ TCID₅₀/ml solutions of MERS-CoV and

FIGURE 1

Viability over time of Middle East respiratory syndrome coronavirus (MERS-CoV) and A/Mexico/4108/2009 (H1N1) virus under different environmental conditions



RH: relative humidity; TCID₅₀: tissue culture infective dose 50.

10⁶ TCID₅₀ of MERS-CoV (isolate HCoV-EMC/2012) (panels A and B) or A/Mexico/4108/2009 (H1N1) virus (panels C and D) was spotted on plastic (panels A and C) or steel (panels B and D) surfaces, incubated at 20°C - 40% RH (blue); 30°C - 30% RH (green) and 30°C - 80% RH (red) and titrated on VeroE6 cells (for MERS-CoV) or Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus).

A/Mexico/4108/2009 (H1N1) virus respectively were aerosolised in triplicate for 10 minutes. Aerosols were collected continuously during aerosolisation in tissue culture media (10 ml, DMEM) with an All Glass Impinger (Ace Glass Inc., USA). Collected aerosols were analysed by quantitative real-time polymerase chain reaction (qRT-PCR) and by virus titration [10,13,14]. TCID₅₀ equivalents were generated using a standard curve of 10-fold diluted MERS-CoV RNA of known concentration in the qRT-PCR. Viral genomic RNA levels in TCID₅₀ equivalents, representative of total amount of virus particles, were compared to TCID₅₀, representative of

the amount of viable virus of either MERS-CoV or A/Mexico/4108/2009 (H1N1) virus [13,14].

MERS-CoV decreased only 7% in viability at 40% RH, whereas the viability at 70% RH decreased significantly with 89% (unpaired one-tailed Student's t-test, $p=0.0045$). The viability of A/Mexico/4108/2009 (H1N1) virus decreased under both conditions with 95% for 40% RH and 62% for 70% RH respectively. This decrease was found to be significant at 40% RH ($p=0.0095$), but not at 70% RH and did not differ significantly between the two conditions.

TABLE

Decay of Middle East respiratory syndrome coronavirus (MERS-CoV) on plastic and steel surfaces at different temperatures and percent humidity

Surface type; temperature, relative humidity	Mean half-life time of MERS-CoV (hours) ^a	Standard deviation
Plastic; 20°C, 40%	0.954523	1.110443
Plastic; 30°C, 30%	0.441822	0.345291
Plastic; 30°C, 80%	0.904005	4.6838
Steel; 20°C, 40%	0.940139	1.837771
Steel; 30°C, 30%	0.973656	0.31109
Steel; 30°C, 80%	0.641163	0.825395

^a Mean half-life was determined from three independent experiments.

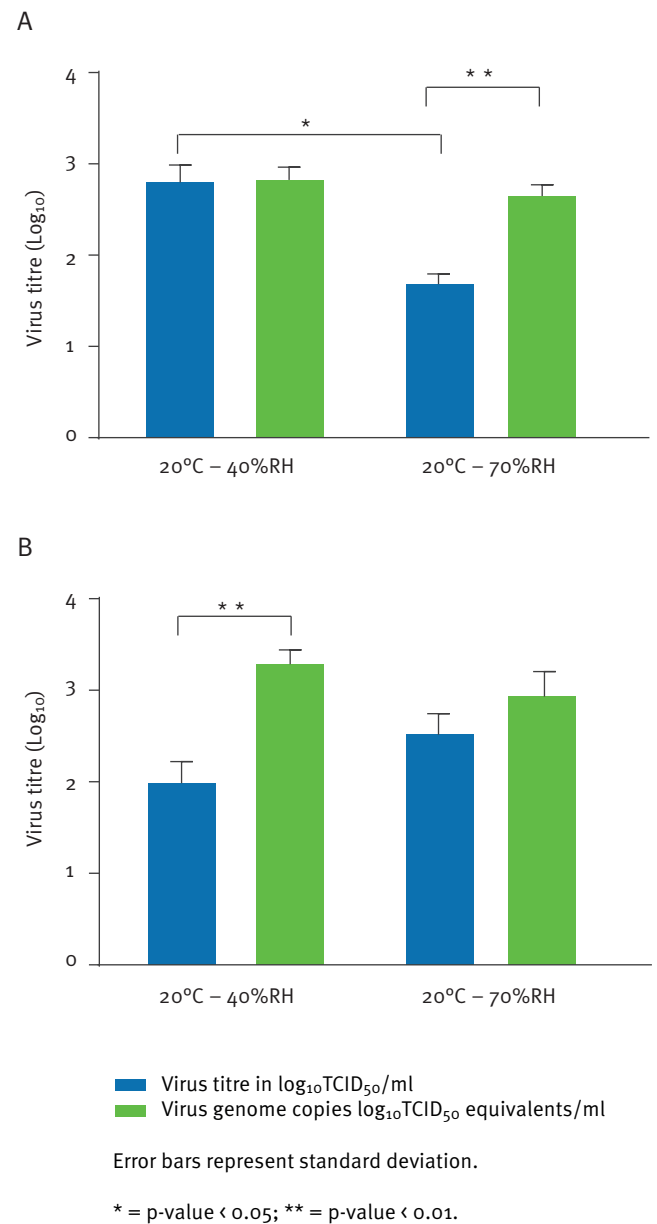
Discussion

Since MERS-CoV emerged [1], an increasing number of human cases have been identified in eight different countries with a case-fatality rate of 50- to 60% [15]. Small clusters of cases with human-to-human transmission have occurred in the United Kingdom, France and Italy. In these clusters, initial cases had a recent travel history to the Middle East and subsequently infected secondary cases [2-5]. In addition, the largest cluster with suspected human-to-human transmission of MERS-CoV has been observed in Saudi Arabia and is epidemiologically linked to healthcare facilities, suggesting nosocomial transmission [16]. The recent identification of the potential circulation of MERS-CoV in dromedary camels could indicate that both zoonotic and human-to-human transmission is involved in the ongoing spread of MERS-CoV [17,18].

Here we show that compared to A/Mexico/4108/2009 (H1N1) virus, MERS-CoV remains viable for a longer duration in the environment. After four hours no viable A/Mexico/4108/2009 (H1N1) virus was detected in comparison to 8, 24 or 48 hours for MERS-CoV depending on environmental conditions (Figure 1, panels A and D). MERS-CoV was very stable in aerosol form at 20°C – 40% RH. The decrease in viability at 20°C – 70% RH (89%) was comparable to that of A/Mexico/4108/2009 (H1N1) virus. Severe acute respiratory syndrome coronavirus (SARS-CoV) has been reported to stay viable for up to five days at 22 to 25°C and 40 to 50% RH and increase in temperature and humidity resulted in a rapid loss of viability [19]. Although a comparison between different experimental studies should be approached cautiously, the relative stability of MERS-CoV at 20°C – 40% RH and the rapid decrease in virus viability at higher temperatures and higher humidity suggests that MERS-CoV and SARS-CoV share relatively similar stability characteristics. Although the route of transmission for MERS-CoV is currently unknown, the spread of MERS-CoV between people in close contact

FIGURE 2

Aerosol stability of Middle East respiratory syndrome coronavirus (MERS-CoV) and A/Mexico/4108/2009 (H1N1) virus under different relative humidity conditions*



TCID₅₀: tissue culture infective dose 50.

10⁶ TCID₅₀/ml of MERS-CoV (panel A) and A/Mexico/4108/2009 (H1N1) (panel B) were aerosolised and viability was determined by titration on VeroE6 cells (for MERS-CoV) or Madin-Darby canine kidney (MDCK) cells (for A/Mexico/4108/2009 (H1N1) virus), and compared to TCID₅₀ equivalents derived by quantitative real-time polymerase chain reaction (qRT-PCR).

TCID₅₀ equivalents were extrapolated from standard curves generated by adding dilutions of RNA extracted from a MERS-CoV stock with known virus titre in parallel to each run.

settings suggest contact and fomite transmission routes are most likely involved [2,3,16]. Knowledge on the environmental stability of MERS-CoV does not provide direct insights in the route of transmission; yet it does provide us with a better understanding for the potential of aerosol, contact and fomite transmission. The prolonged survival of MERS-CoV compared to A/Mexico/4108/2009 (H1N1) virus on surfaces increases the likelihood of contact and fomite transmission. However, the decrease in viability observed at high temperature suggests that direct contact transmission, and not fomite transmission, in the Arabian Peninsula would be the most likely route of zoonotic and human-to-human transmission in outdoor settings. The ability of MERS-CoV to remain viable in an airborne state suggests the potential for MERS-CoV to acquire the ability to be transmitted via aerosols. In the absence of therapeutic and prophylactic intervention strategies for MERS-CoV, a thorough understanding of the routes of transmission could be the most effective way to arrest the further spread of MERS-CoV.

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Conflict of interest

None declared.

Authors' contributions

N.v.D. and V.J.M. conceived and designed the study, N.v.D. and T.B. performed the experiments, N.v.D. and V.J.M. analysed the data, N.v.D. and V.J.M wrote the manuscript.

*Erratum

Figure 2 was corrected and replaced on 23 September 2013.

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Whole genome sequencing and phylogenetic analysis of West Nile virus lineage 1 and lineage 2 from human cases of infection, Italy, August 2013

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A human outbreak of West Nile virus (WNV) infection caused by WNV lineage 2 is ongoing in northern Italy. Analysis of six WNV genome sequences obtained from clinical specimens demonstrated similarities with strains circulating in central Europe and Greece and the presence of unique amino acid changes that identify a new viral strain. In addition, WNV lineage 1 Livenza, responsible for a large outbreak in north-eastern Italy in 2012, was fully sequenced from a blood donor during this 2013 outbreak.

A human outbreak of West Nile virus (WNV) infection is ongoing in northern Italy. We report here the clinical presentation, laboratory test results, and WNV full genome sequences from human cases of WNV infection which were diagnosed in August 2013 in the Veneto region. This region is one of the most affected by the outbreak. WNV genome sequences obtained from clinical specimens of infected individuals were subjected to phylogenetic analysis, in order to gain information on the origin and evolutionary history of the responsible viral strains.

Cases of West Nile virus infection from the Veneto region

Cases included five patients with laboratory-confirmed West Nile neuroinvasive disease (WNND) and four with West Nile fever (WNF), aged 51 to 88 years-old, who were resident in Rovigo, Padova, and Verona provinces (Figure 1). In addition, three WNV RNA-positive blood and organ donors were identified by screening in Padova, Verona, and Venice provinces and, besides the 12 confirmed cases, further possible cases are currently under investigation. Confirmed human cases of WNND have also been notified in regions neighbouring Veneto, namely Emilia Romagna and Lombardy [1].

Of the 12 confirmed cases reported in Veneto, WNV lineage 2 (lin2) RNA was identified in plasma and/or urine

of seven patients with WNND or WNF, and in a blood donor, while WNV lin1 was respectively detected in an organ donor and in a blood donor. The sites where different WNV lineages were identified are indicated in Figure 1.

Clinical and laboratory findings

A summary of clinical and laboratory findings from confirmed cases is reported in Table 1. Clinical presentation of patients with WNND and WNF included arthralgia, fatigue, fever ($\geq 38^{\circ}\text{C}$), headache, myalgia, while patients with WNND had neurological manifestations, such as encephalitis, meningitis and paralysis. Mild symptoms (i.e. arthralgia, headache, myalgia, but not fever) occurred also in a WNV-positive blood donor a few days before donation. No deaths due to WNV infection were reported.

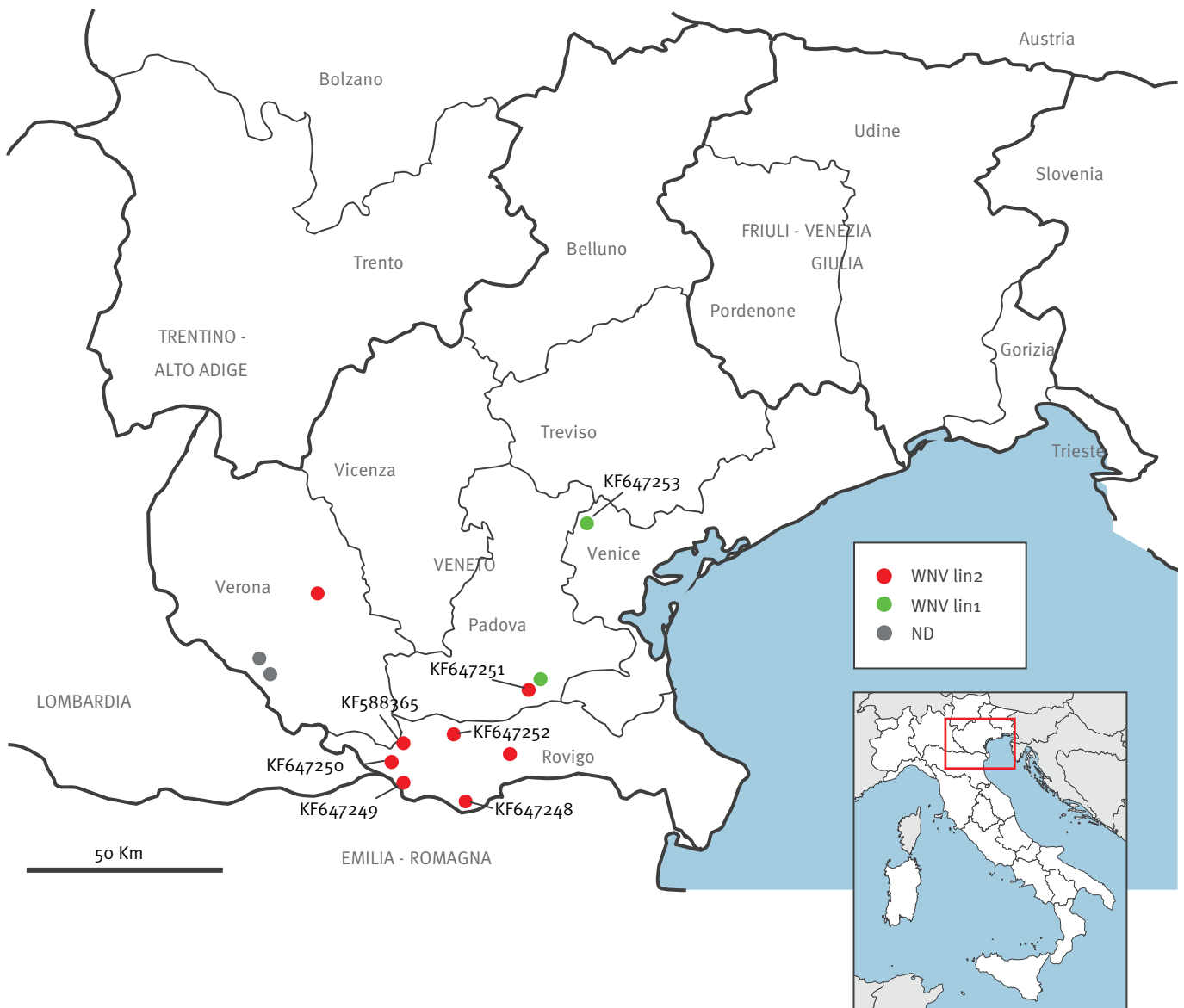
Isolation of the virus in cell cultures was obtained from urine samples collected from three patients with WNND or WNF and from two blood donors. Laboratory methods were performed as previously described [2].

Epidemiological situation of West Nile virus infection in Europe and Italy

Since 2010, WNV, a mosquito-borne flavivirus, has become a public health concern in Europe, as it has been responsible for an increasing number of epidemic outbreaks in European countries and in neighbouring countries in the Mediterranean basin as well as in the Russian Federation [3]. In fact, after large human outbreaks with hundreds of cases of neuroinvasive disease (WNND) occurred in Romania in 1996 and in Russia in 1999, only small outbreaks were reported in European and Mediterranean countries, generally caused by WNV lin1 strains [4]. The epidemiological situation in Europe changed in 2010, when two large human outbreaks occurred in Greece [5] and in the Volgograd region, Russia [6,7]. During these two outbreaks, two

FIGURE 1

Map showing the places of residence of human cases of West Nile virus (WNV) infection confirmed in the Veneto region, Italy, August 2013 (n=12)



ND: WNV case whereby WNV lineage was not determined; WNV lin1: WNV case with WNV lineage 1 infection; WNV lin2: WNV case with WNV lineage 2 infection.

GenBank accession numbers of WNV genome sequences are indicated near the corresponding case symbols.

unrelated WNV lin2 genotypes were respectively characterised. The genotype in the Greek outbreak (Greece-Nea Santa-2010) [8] was similar to that first found in a goshawk in Hungary in 2004 (Hungary04 strain) [9] while in the Volgograd outbreak the genotype was similar to that prior detected in human brain and blood samples in 2007 in Volgograd [6]. Recent epidemiological data indicate that WNV lin2 of the Greek/Hungarian cluster is spreading to other central and southern European countries [10,11], such as Serbia, where a large human outbreak occurred in 2012 [7] and is ongoing in 2013 [1].

The outbreak described in this report represents the first human outbreak of WNV lin2 infection reported in Italy. In fact, in Italy, most cases of human infection reported before 2013 were caused by WNV lin1. In particular, two unrelated WNV lin1 strains, both classified within the Mediterranean cluster by phylogenetic analysis, had been responsible for two different outbreaks in northern Italy. The first outbreak between 2008 and 2009 was caused by the WNV lin1 Italy 2008–2009 strain [12–14] and occurred in the same areas that are currently affected by WNV lin2 circulation, namely those surrounding the Po river in the Veneto, Emilia-Romagna, and Lombardy regions [12,15]; the second

TABLE 1

Clinical and laboratory data of laboratory-confirmed cases of West Nile virus infection, Veneto region, Italy, August 2013 (n=12)

Case	Time to diagnosis (days) ^a	Symptoms	Laboratory findings ^b	WNV RNA load in plasma (GE/mL)	WNV RNA load in urine (GE/mL)	Type of sample where WNV was isolated	WNV genome sequencing (GenBank name, accession number, type of sample)	WNV lineage
WNND	3	Fever, meningitis, upper limbs' paralysis	WNV RNA in plasma and urine, WNV IgM+/IgG- in serum and CSF, viral isolation in culture	1,300	8,300,000	Urine	Italy/2013/Rovigo/32.1, KF588365; urine	2
WNND	12	Arthralgia, encephalitis, fever, headache	WNV RNA in plasma, WNV IgM+/IgG- in serum and CSF	190,000	NA	WNV not isolated	Italy/2013/Rovigo/34.1, KF647248; plasma	2
WNND	2	Fever, meningitis,	WNV RNA in urine, WNV IgM+/IgG+ in serum and CSF, viral isolation in culture	Undetectable	15,000,000	Urine	Italy/2013/Padova/34.1, KF647251; urine	2
WNND	4	Fever, encephalitis, headache	WNV RNA in plasma and urine, WNV IgM+/IgG+ in serum	100	1,300,000	WNV not isolated	Italy/2013/Rovigo/35.1, KF647252; urine	2
WNND	2	Fatigue, fever, encephalitis, vomiting, rash	WNV RNA in plasma and urine, WNV IgM+/IgG- in serum and CSF	400	350,000	WNV not isolated	ND	2
WNF	21	Arthralgia, fatigue, fever, myalgia	WNV RNA in urine, WNV IgM+/IgG+ in serum	Undetectable	350	WNV not isolated	ND	ND
WNF	10	Arthralgia, fatigue, fever, headache, myalgia	WNV RNA in urine, WNV IgM+/IgG- in serum	Undetectable	1,200,000	WNV not isolated	Italy/2013/Rovigo/33.1, KF647250; urine	2
WNF	8	Arthralgia, fatigue, fever, headache, rash	WNV RNA in urine, WNV IgM+/IgG- in serum, viral isolation in culture	Undetectable	180,000	Urine	Italy/2013/Rovigo/33.2, KF647249; urine	2
WNF	14	Arthralgia, fatigue, fever, headache, myalgia	WNV IgM+/IgG+ in serum	Undetectable	Undetectable	WNV not isolated	ND	ND
Organ donor	0	NR	WNV RNA in plasma	3,800	NA	NA	ND	1
Blood donor	5	Asymptomatic	WNV RNA in plasma and urine, WNV IgM-/IgG-, viral isolation in culture	3,900	1,000	Urine	Italy/2013/Livenza/35.1, KF647253; urine	1
Blood donor	4	Arthralgia, headache, myalgia	WNV RNA in plasma and urine, WNV IgM-/IgG-, viral isolation in culture	50,000	3,200	Urine	ND	2

CSF: cerebrospinal fluid; GE: genome equivalents; NA: sample not available; ND: not determined; NR: not reported; WNF: West Nile fever; WNND: West Nile neuroinvasive disease; WNV: West Nile virus.

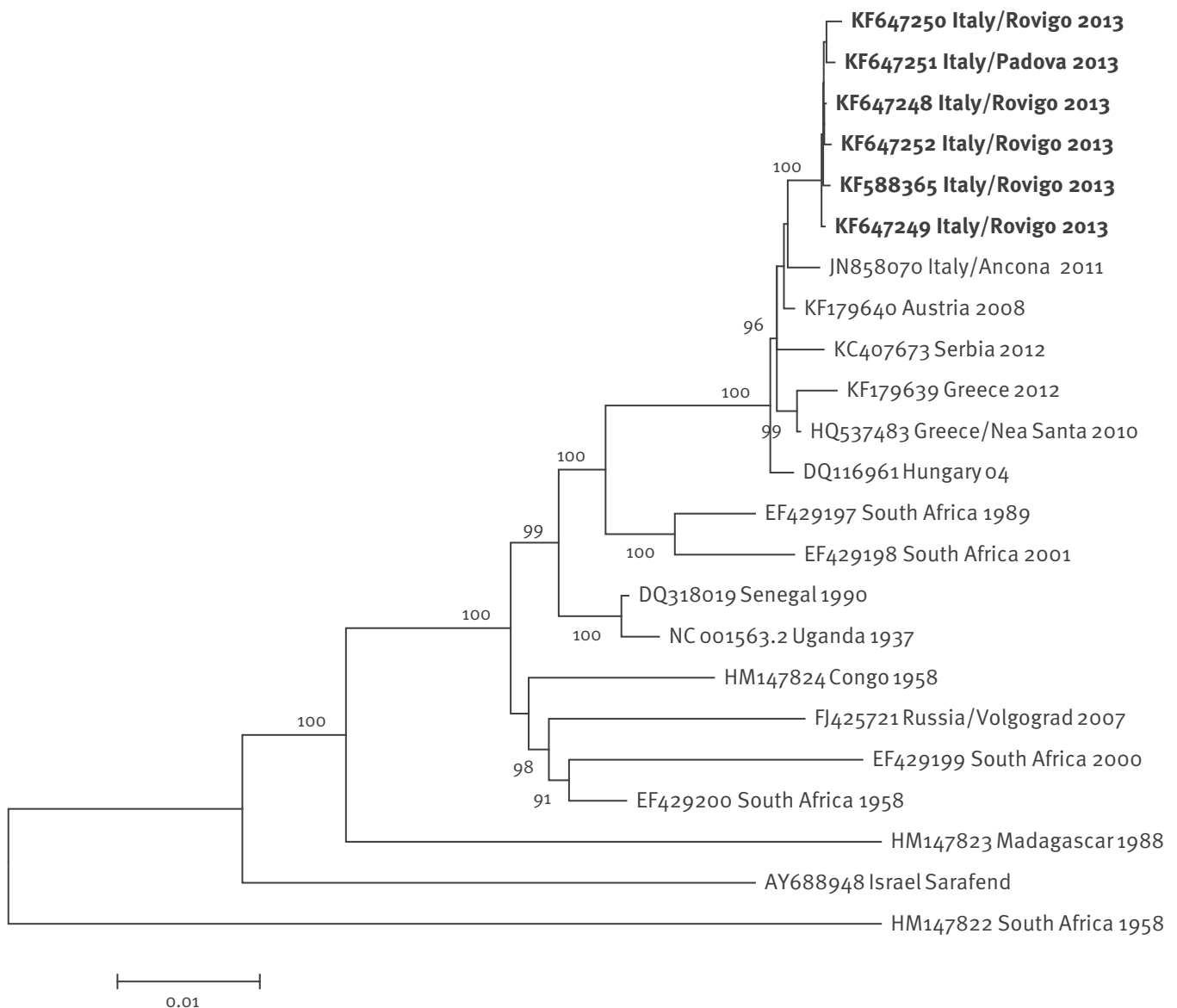
Fever is determined as body temperature $\geq 38^{\circ}\text{C}$.

^a This is the time interval between symptom onset or blood/organ donation (for blood or organ donors) and diagnosis.

^b WNV RNA was detected by real-time reverse-transcription polymerase chain reaction; WNV IgM and IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA) and confirmed by plaque reduction neutralisation assay.

FIGURE 2

Molecular phylogenetic analysis of six West Nile virus lineage 2 genome sequences detected in individuals from the Veneto region, Italy, August 2013



Sequences detected in individuals from the Veneto region are shown in bold font. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [25]. The bootstrap consensus tree inferred from 1,000 replicates [26] is taken to represent the evolutionary history of the taxa analysed [26]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (values ≥ 80) [26]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 23 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 8,586 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [27].

outbreak took place between 2011 and 2012 in the Venice and Treviso provinces of the Veneto region and was caused by the WNV lin1 Livenza strain [16,17].

Before 2013 only two unrelated human cases of WNV lin2 infection representing the Greek/Hungarian cluster were documented in the country. These had occurred in 2011 and included one case in Ancona (Marche region) and one in Olbia (Sardinia region), respectively [18,19]. In 2011 and 2012, however, WNV lin2 belonging to the

Greek/Hungarian cluster was detected by entomological and veterinary surveillance in the island of Sardinia as well as in the Veneto and Friuli-Venezia Giulia regions in north-eastern Italy in areas where WNV lin1 was also circulating [20-22].

The fact that most human cases from Veneto in August 2013 are affected by WNV lin2 could suggest that this lineage has now become more widespread in north-eastern Italy and that it is playing an important part in

TABLE 2

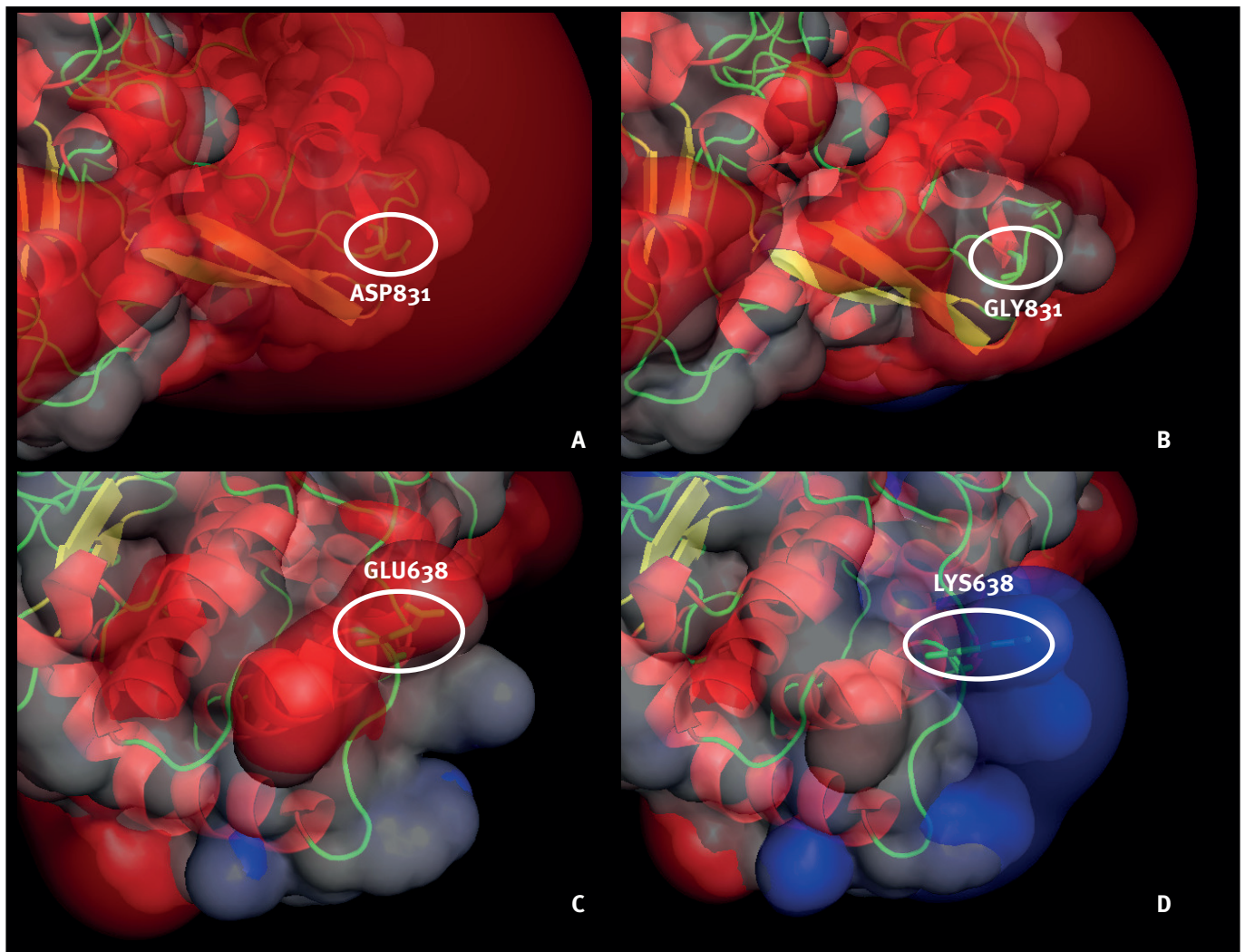
Variant amino acids encoded by West Nile virus (WNV) lineage 2 genome sequences, including the reference Hungary 2004 sequence, other related sequences, and six genome sequences obtained during the WNV outbreak in Italy, August 2013

Genome sequence name GenBank accession number	Mature peptide Amino acid position in mature peptide (in polyprotein)																													
	PrM	M	E			NS1			NS2A	NS2B	NS3	NS4A	NS4B			NS5														
Hungary 04 DQ116961	A	T	I	S	I	V	L	G	A	A	H	S	V	Q	H	I	V	S	T	V	A	Y	G	A	N	R	E	T	D	885 (3414)
Italy/2013/ Rovigo/32.1 KF588365	A	A	I	P	T	V	L	E	V	A	Y	C	V	Q	H	I	V	S	T	V	T	H	G	T	S	R	K	T	D	A
Italy/2013/ Rovigo/33.1 KF647250	A	A	I	P	T	V	L	E	V	A	Y	C	V	Q	H	I	V	S	T	V	T	H	G	T	S	R	E	T	G	A
Italy/2013/ Padova/34.1 KF647251	A	A	M	P	T	G	S	E	V	A	Y	C	V	Q	H	I	V	S	T	V	T	H	G	T	S	R	E	T	D	A
Italy/2013/ Rovigo/34.1 KF647248	A	A	I	P	T	V	L	E	V	A	Y	C	-	Q	H	I	M	S	T	V	T	H	G	T	S	R	E	T	D	A
Italy/2013/ Rovigo/33.2 KF647249	A	A	I	P	T	V	L	E	V	A	Y	C	I	Q	H	I	V	S	T	V	T	H	G	T	S	R	E	T	D	A
Italy/2013/ Rovigo/35.1 KF647252	A	A	I	P	T	V	L	E	V	A	Y	C	V	Q	H	I	M	S	T	V	T	H	G	T	S	R	E	T	D	A
Austria 2008 KF179640	A	T	I	P	I	V	L	E	A	A	Y	S	V	Q	H	I	V	S	T	V	T	Y	G	A	N	R	E	T	D	V
Greece-Nea Santa-2010 HQ537483	A	T	I	P	I	V	L	E	A	A	Y	S	I	Q	P	I	V	G	A	M	T	Y	G	A	N	R	E	T	D	V
Italy-AN-2 2011 JN858070	V	T	I	P	T	V	L	E	A	V	Y	S	V	Q	H	T	V	S	T	V	T	Y	S	T	N	H	E	A	D	V
Serbia 2012 KC407673	A	T	I	P	I	V	L	E	A	A	Y	S	V	H	H	I	V	S	T	V	T	Y	G	A	N	R	E	T	D	V

E: envelope protein; M: membrane protein; NS: non-structural protein; PrM: pre-membrane protein. Amino acids that are unique in WNV lineage 2 genomes from Italy 2013 are highlighted in red. (-) indicates missing sequence information in partially sequenced genome.

FIGURE 3

3D images of mutations in the RNA-directed RNA polymerase domain of West Nile virus non-structural protein 5 (NS5)



Panels A and B show a mutation from ASP831 to GLY831 and panels C and D show a mutation from GLU638 to LYS638.

The 3 D images are predicted by *in silico* site-directed mutagenesis based on the Protein Data Bank (PDB) 2HCN template [28] Best rotamer of side chain of LYS638 has been placed in place of corresponding GLU638 in 2HCN template. GLY831 has been obtained by removing the side chain of corresponding ASP831 in 2HCN template. Structures rendering has been obtained by Pymol 1.5.0.3 (<http://www.pymol.org>). Per-atom charge and radius have been calculated by CHARMM force field using pdb2pqr tool (<http://www.poissonboltzmann.org/pdb2pqr> [29,30]). Molecular surface is coloured according to the potential on solvent accessible surface and has been calculated using APBS tool (<http://www.poissonboltzmann.org/apbs> [31]) for Poisson-Boltzmann electrostatics and shown in the range of -5/+5 kT/e.

Negative isosurface and positive isosurface are shown with a gradient that goes from red (-2 kT/e) to blue (+2 kT/e) respectively.

GLY831 weakens the negative surface (see panel B compared with panel A) whereas LYS638 contributes to change the sign of the surface charge from negative to positive (see panel C compared with panel D).

the current human outbreak. To gain more insight into the origin of the WNV lin2 and WNV lin1 involved in this ongoing outbreak, respective genome sequences were sought.

Genome sequences derived from the 2013 West Nile virus outbreak cases in Veneto

A total of seven WNV lin2 and one WNV lin1 genome sequences were derived from blood or urine samples of cases, including two full genome sequences of WNV

lin2 sequenced from samples collected at three days-interval from the same patient. Six WNV complete genome sequences and one almost complete were submitted to GenBank with accession numbers KF588365 and KF647248–KF647253.

Analysis of the West Nile virus lineage 1 sequence
Sequencing of the full genome of WNV lin1 detected in a blood donor from Venice province (i.e. Italy/2013/Livenza/35.1, GenBank accession number: KF647253)

demonstrated over 99.9% nucleotide sequence identity with the Livenza strains fully sequenced in 2011 and 2012 and responsible for the large human outbreak that occurred in Venice and Treviso provinces in 2012 [16,17]. This finding demonstrates that the Livenza strain is still circulating in the affected area.

Phylogenetic and amino acid analyses of West Nile virus lineage 2 sequences

Sequence alignment demonstrated that all the genome sequences derived from the cases infected with WNV lin2 (WNV lin2 Italy/2013) shared over 99.9% nucleotide sequence identity, and the two WNV lin2 genome sequences derived from the same patient had 100% identity. At variance, the identity with other WNV lin2 genomes was lower, e.g. 99.5% vs the WNV lin2 Hungary04 strain [9] and 99.4% vs a WNV lin2 Greece-Nea Santa-2010 strain [8]. Likewise 99.7% and 99.1% nucleotide sequence identities were also respectively observed with the NS3 and NS5 regions of a WNV lin2 detected in a mosquito pool collected in 2012 in Rovigo province, in the same area of the current outbreak [21]. Finally the present outbreak sequences presented 99.5% nucleotide sequence identity with the full genome of the WNV lin2 isolated from the patient in Ancona in 2011 [19]. Phylogenetic analysis showed that the WNV lin2 Italy/2013 genomes were included in the Greek/Hungarian cluster which contains the Hungarian (Hungary04) and Greek (Greece-Nea Santa-2010) strains, but generated a distinct branch in the phylogenetic tree, indicating that they represent a new strain (Figure 2). This finding suggests that a single monophyletic group of WNV lin2 is arising from the Greek/Hungarian cluster, which corresponds to a group of viruses that are evolving as they reach new territories in their spread from central Europe and areas in the Balkans.

At protein level, the WNV lin2 Italy/2013 genomes encoded a set of unique amino acids compared to other fully sequenced WNV lin2 genomes of the Greek/Hungarian cluster (Table 2). Most of the substitutions compared to the Hungary 04 reference apparently seem not to change dramatically the properties of referring proteins. Nonetheless, mutations observed on the surface of the non-structural protein 5 (NS5) protein (i.e. E638K* and D831G) in two individual genome sequences were predicted by *in silico* site-directed mutagenesis to cause a local altered electrostatic potential in the RNA-directed RNA polymerase domain (Figure 3). The relevance of these mutations will be assessed by further sequencing of WNV genome sequences and by experimental studies with viral isolates and site-directed mutagenesis of infectious clones. Of note, none of the WNV lin2 Italy/2013 genomes had the H249P substitution in non-structural protein 3 (NS3) that characterises the Greece-Nea Santa-2010 strain.

Conclusion

Overall, the results of this molecular epidemiology study shows that genetically different lineages of WNV

are capable of establishing in Europe, remain circulating for several years in the same territory, and spread slowly to neighbouring areas, in agreement with other reports from Europe [13,23]. In this local transmission and spread dynamics in Europe, WNV overwintering in mosquitoes and amplification in local susceptible bird populations are key factors, while WNV re-introduction by migrating birds from long-distance Euro-African routes seems to be less relevant [24].

In conclusion, a novel WNV lin2 strain of the Greek/Hungarian cluster is responsible for a human outbreak of neuroinvasive disease that is ongoing in northern Italy. The virus is co-circulating with the WNV lin1 Livenza strain that caused a large human outbreak in 2012.

*Authors' correction:

At the request of the authors, 'E636K' was replaced with 'E638K'. This change was made on 27 September 2013.

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Conflict of interest

None declared.

Authors' contributions

Luisa Barzon coordinated the study and wrote the manuscript; Monia Pacenti, Riccardo Cusinato, Silvana Pagni, Margerita Cattai, and Laura Squarzon performed surveillance activities and virological tests; Elisa Franchin and Giulia Masi performed WNV genome sequencing; Enrico Lavezzo and Stefano Toppo performed bioinformatics analyses of WNV genome sequences; Francesca Russo coordinated WNV surveillance activities; Giorgio Palù supervised the study and revised the manuscript.

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Pertussis immunisation and control in England and Wales, 1957 to 2012: a historical review

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This review summarises the epidemiology and control of pertussis in England and Wales since the introduction of routine immunisation and considers the implications for future control. Routine infant immunisation with a whole-cell pertussis (wP) vaccine was introduced in 1957 and had a marked impact on the overall disease burden. Following a fall in vaccine coverage during the 1970s and 80s linked to a safety scare with wP vaccine, there was an extended period of high coverage and pertussis incidence fell dramatically. Incidence continued to decrease with the introduction of an acellular pertussis vaccine in the pre-school booster in November 2001 and in the primary United Kingdom (UK) schedule in September 2004 but has increased since July 2011. In response to a high rate of pertussis in infants, a temporary vaccination programme for pregnant women was introduced in October 2012. The key aim of the programme is to protect vulnerable infants from birth in the first months of life, before they can be fully protected by routine infant immunisation. A review of the UK adolescent immunisation programme is currently ongoing and the inclusion of a pertussis booster is being considered.

Introduction

Pertussis (whooping cough) is an acute bacterial respiratory infection caused by *Bordetella pertussis*. It is characterised by a protracted coughing illness that can last for several weeks. The illness typically begins with a catarrhal stage followed by periods of intense paroxysmal coughing spells. Infants under 1 year are at the highest risk of complications, such as pneumonia and seizures, while adolescents and adults tend to display milder symptoms, sometimes without the classic features of a protracted paroxysmal cough.

Humans are the only known host for *B. pertussis*, so elimination through vaccination is theoretically possible. However, protection conferred by natural infection and current vaccination schedules is not lifelong [1]. Routine pertussis immunisation was introduced in England and Wales in 1957 with a whole-cell pertussis (wP) vaccine for infants from 3 months of age. In the pre-vaccine era, large epidemics of pertussis occurred

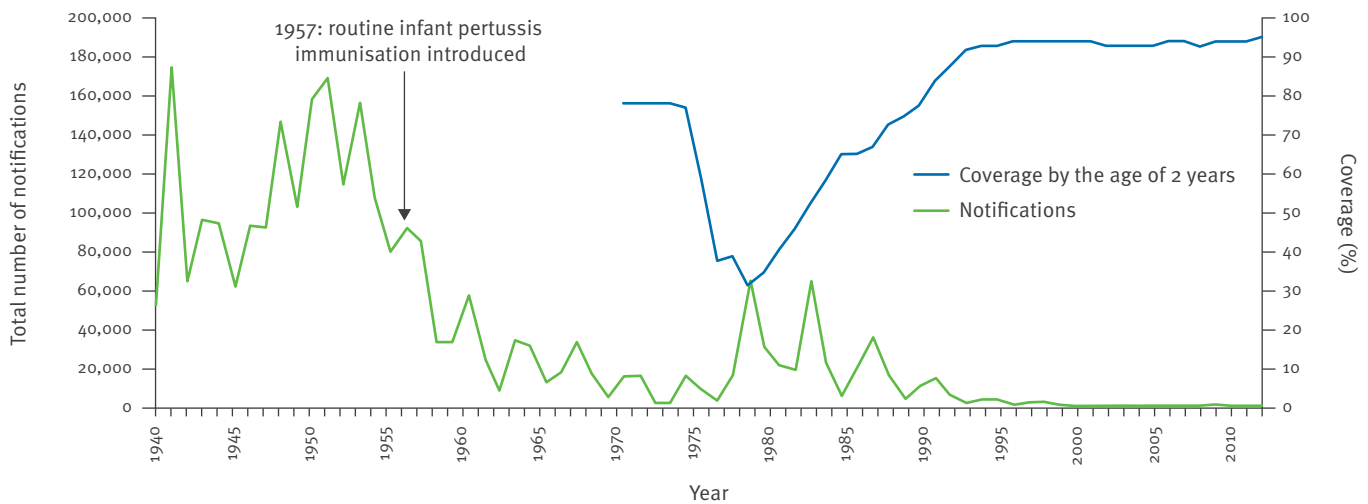
every three to five years, affecting up to 150,000 people and contributing to approximately 300 deaths each year [2]. Although England and Wales have experienced an extended period of high vaccine coverage and disease incidence has fallen dramatically, pertussis remains the most common vaccine-preventable cause of hospitalisation and death in infants [3].

Pertussis persists as an infection of global public health importance. Many countries with long-standing vaccination programmes have reported a resurgence of pertussis, particularly in adolescents and adults [4-6] and young infants less than 6 months of age [7-9], despite sustained high vaccine coverage. This has led to a growing international debate on the potential strategies to optimise global pertussis control. A 2010 review by the Strategic Group of Experts in Immunisation (SAGE) on pertussis control strategies recommended a booster dose for children aged 1-6 years, preferably during the second year of life, following completion of the primary infant schedule [10]. Although a number of countries, including France, the United States (US) and Australia, have recommended adolescent boosters and cocooning (vaccinating close household contacts of young infants), data to support the introduction of neonatal pertussis immunisation remain inconclusive [10]. In 2011, the US became the first country to advise that pertussis-containing vaccine can be safely administered to pregnant women who have not previously received the recommended adult dose [11]. This advice was updated in October 2012 to recommend that pertussis-containing vaccine be routinely offered to women in every pregnancy [12].

Understanding the impact of different control strategies on pertussis epidemiology is likely to inform future vaccine policy in the UK and other countries. In this review, we present a historical overview of the epidemiology and control of pertussis in England and Wales in the pre- and post-vaccine era and the implications for future pertussis control.

FIGURE 1

Annual notifications of pertussis (1940–2012, England and Wales) and vaccine coverage by the age of 2 years (1970–2012, England only)



Source: [3], updated with data up to 2012.

Methods

Searches, not limited by language or country, were undertaken in November 2011 with the terms *Bordetella pertussis*, whooping cough, epidemiology and pertussis vaccine using the Cochrane Library, MEDLINE (1950–2011), the Cumulative Index to Nursing and Allied Health Literature (CINAHL) (1981–2011), Embase (1980–2011), the International Standard Randomised Controlled Trial Number (ISRCTN) Register, the Health Management Information Consortium database (a compilation of data from two sources, the UK Department of Health Library and Information Services and the King's Fund Information and Library Service) and from bibliographies of collected papers and reviews. An updated search was undertaken in September 2013, prior to publication, to identify any further relevant papers.

Grey literature and unpublished surveillance data from England and Wales were also identified and a number of key UK sites, including the Department of Health, Office for National Statistics and Health Protection Agency (now Public Health England), were accessed. Epidemiological data for England and Wales up to December 2012 were extracted. International websites were also searched, including those of the World Health Organization, the US Centers for Disease Control and Prevention and the Australian Department of Health and Ageing. All the abstracts or the full paper, if no abstract was available, were reviewed for relevance. Studies were included if any of the following criteria were met, based on a review of the full paper: epidemiological data on pertussis in England and Wales; pertussis vaccine trials and pertussis vaccine policy in England and Wales.

Results

Literature search

Around 650 articles were retrieved and each was designated to one of the three authors and the abstracts reviewed for relevance. When the abstract was not available, the full paper was obtained and assessed for a decision on its inclusion. Approximately 320 full papers were reviewed for inclusion. Following author review, 76 papers were retained for inclusion in the final review.

Development of the whole-cell vaccine and early vaccine trials (1913–1957)

The French researchers Bordet and Gengou described *B. pertussis* as the causative agent of whooping cough in 1906 [13]. By the 1920s, scientists had developed vaccines to control many infectious diseases including smallpox, typhoid fever, diphtheria and tetanus, but pertussis proved more challenging. By the 1930s, pertussis was an increasingly dominant cause of childhood mortality, outranking diphtheria, scarlet fever and measles in many European countries [14]. During the 1930s, the American researchers Kendrick and Eldering performed animal studies to design a potential candidate vaccine using inactivated whole *B. pertussis* bacilli [15–18]. In a field trial involving 1,592 (712 vaccinated and 880 control) children, there were 63 pertussis cases in the control group and only three in the vaccinated group [15]. North-American field trials of newer inactivated vaccines conducted in the 1940s and 50s also demonstrated a protective effect [15,19–21]. As a result, the American medical community began to use the pertussis vaccine on an ad hoc basis [22].

TABLE

Pertussis in England and Wales, 1944–1973

Years	Incidence			Case fatality			Mortality		
	Notifications per 1,000 population			Deaths per 1,000 notifications			Deaths per million population		
	<1 year	1–4 years	5–9 years	<1 year	1–4 years	5–9 years	<1 year	1–4 years	5–9 years
1944–45	12.1	17.7	8.4	65.7	6.99	1.05	796.3	123.6	8.8
1946–49	14.6	22.4	11.0	42.6	4.07	0.40	620.6	91.0	4.4
1950–53	19.3	28.2	15.0	15.9	1.24	0.12	307.7	35.1	1.8
1954–57	12.1	16.5	9.7	8.8	0.55	0.09	106.1	9.0	0.8
1958–61	5.0	6.1	4.4	5.3	0.46	0.07	26.5	2.8	0.3
1962–65	3.1	3.5	2.1	9.2	0.60	0.04	28.2	2.1	0.1
1966–69	2.3	3.0	1.6	8.7	0.13	ND	19.8	0.4	ND
1970–73	1.6	1.3	0.9	8.2	0.24	0.14	13.1	0.3	0.1

ND: no deaths.

Source: [2].

Meanwhile, the vaccine was not widely accepted in the UK. The UK Medical Research Council (MRC) trials (1942–1944) had shown such vaccines to be ineffective [23] and the editor of the *British Medical Journal* cited these findings and argued that none of the American studies used proper control groups [24]. The Whooping Cough Immunisation Committee in the UK concluded that the vaccines used in the MRC trials differed in an undetermined way from those vaccines shown to offer protection in the American and Canadian studies [25] and so performed new field trials [26,27] with a number of vaccines, including those of American origin used in previous studies.

In 1956, a review of two series of field studies was published in which 14 pertussis vaccines were tested for their effectiveness in 28,799 children [26]. The vaccines used in the first series of trials gave poor protection while those in the second series showed protection that was maintained for up to three years [28]. The latter series included three UK-manufactured vaccines using endemic strains of *B. pertussis* with comparable effectiveness to the reference vaccine made with American strains [26]. The studies highlighted the variability in the protection afforded by pertussis vaccines at that time. General and severe local reactions were rare.

While the field trials were in progress, vaccines were tested for their ability to protect mice against intra-cerebral pertussis infection [26]. A comparison between field and animal studies showed some correlation between the effectiveness in children and the ability to protect mice against intra-cerebral infection. The MRC trial concluded that only those vaccines with adequate efficacy, as demonstrated by the intra-cerebral mouse-protection test, should be issued for use in children [26].

Impact of routine wP vaccination in England and Wales
Pertussis first became notifiable in England and Wales in 1940. The annual notifications due to pertussis (1940–2012) and vaccine coverage at 2 years of age (1970–2012) are shown in Figure 1. Despite an overall fall in notifications, a substantial increase in the number of cases was observed from 1948 to 1954. This might have partly been attributable to the post-war population ‘boom’ and increased public and professional awareness of pertussis, as a result of the MRC trials, resulting in more complete notification of clinical cases.

Following the introduction of routine pertussis immunisation with diphtheria, tetanus and whole-cell pertussis (DTwP) vaccine in the UK in 1957, there was a substantial decrease in notifications. Mortality associated with pertussis declined considerably after 1945 (Table) [2]. Before routine immunisation, this decline was observed in children aged under 10 years and so was likely to have been due to improved treatment, including widespread use of antibiotics [2]. Most pertussis-related deaths occurred in infants (Table). Pertussis mortality rates fell further following the introduction of routine immunisation in 1957 (from 106.1 to 13.1 per million infants in 1954–57 and 1970–73, respectively) but case-fatality rates in infants remained relatively constant (5.3–9.2 deaths per 1,000 notifications) during the same period.

In a report published in 1977, there was a consensus within the UK Joint Committee on Vaccination and Immunisation that the introduction of routine pertussis immunisation had been a major factor in the decrease of notified pertussis [2]. This reduction did not continue, however, and notifications stabilised during the 1960s, with sustained 3–4 yearly cycles of increased incidence (Figure 1). The lack of a continued reduction

in pertussis was considered likely to be due to either a change in the infecting bacterium or the use of vaccines with suboptimal effectiveness during 1957 to 1968 [2]. Moreover, considerable concern was raised by members of the committee, manufacturers, local health boards and general practitioners (GPs) regarding the risk of reactions attributable to the pertussis vaccine component. In 1964, the committee evaluated the benefits and risks and concluded that the pertussis vaccine had a protective effect [2]. However, ongoing concerns led to an investigation in 13 areas of Scotland, England and Wales and a Joint Committee on Vaccination and Immunisation report in 1968 revealed that of those children who developed pertussis, 55% were unvaccinated and 42% were vaccinated [29]. A 1969 Public Health Laboratory Service survey showed that vaccines used before 1968 were not very effective, with vaccine effectiveness of 20–30% [30]. Since 1968, only vaccines with a potency of ≥ 4 international units per dose have been marketed and this, together with the addition of aluminium hydroxide adjuvant, led to increased potency of the adsorbed vaccine [31].

Notifications reached a record low of just over 2,000 cases in 1972 (Figure 1). However, there was continuing debate about the impact of pertussis vaccination on disease burden, the risk of severe local and neurological reactions and difficulties with diagnosis, which culminated in a comprehensive review in 1977 [2]. This concluded that the effectiveness had been restored following changes to the vaccine's composition in line with the international potency standards and that a full course reduced both the risk and the severity of the disease.

wP vaccine safety scare in England and Wales (1970–1980s)

Before 1970, reviews of the pertussis vaccine were chiefly focused on its effectiveness. Concern regarding the reactogenicity of the pertussis component of the DTwP vaccine had been expressed for a number of years [32] and a causal relationship with neurological complications was suggested in 1974 [33], which led to a publicity campaign by groups seeking to highlight the hazards of vaccination. Media speculation on the safety of pertussis vaccine stimulated further parliamentary and public interest. In a House of Commons debate in 1974, it was stated that between 1,000 and 2,000 children in the UK had suffered irreversible brain damage as a result of the vaccine [32]. At that time, some publications also suggested that the introduction of mass vaccination had no effect on disease burden and had an association with neurological complications [34]. These findings were in contrast to those of Miller and Fletcher, who reported that the risk of an unvaccinated child being admitted to hospital with pertussis was eight times higher than that of a fully vaccinated child [35].

With sustained adverse publicity, the acceptance rate for pertussis immunisation in England fell from 79%

in 1973 to 31% in 1978 (Figure 1) and the first of three national epidemics of pertussis occurred from 1977 to 1979 [36]. An estimated 5,000 hospital admissions, 200 cases of pneumonia, 83 cases of convulsions and 38 deaths occurred and the illness was often protracted and debilitating, lasting up to 10–12 weeks [23]. In comparison with previous epidemics, the attack rate in children aged under 5 years (in whom coverage was low) was considerably higher. The attack rate was especially high in areas where vaccination uptake was lowest [23].

In 1981, the National Childhood Encephalopathy Study (NCES), which aimed to provide an accurate estimate of the risk of neurological complications following pertussis immunisation, reported a preliminary risk estimate of approximately 1 in 310,000 (95% confidence interval (CI): 1 in 5,310,000 to 1 in 54,000) for a previously healthy child developing neurological sequelae persisting one year after immunisation [37]. However, a Joint Committee on Vaccination and Immunisation review in 1981 concluded that any risk from immunisation was low and outweighed by its advantages and that pertussis vaccine should continue to be recommended as part of the routine UK childhood immunisation programme [23].

An additional Public Health Laboratory Service study in 1982 [38] demonstrated that the vaccine was effective in preventing serious disease, which began to restore confidence in the vaccine. In 1988, the UK High Court ruled that a causal link between the vaccine and permanent brain damage had not been proven [39]. In 1993, the final report from the National Childhood Encephalopathy Study concluded that DTwP vaccine may, on rare occasions, be associated with the development of severe acute neurological illnesses that could have serious sequelae but concluded that the balance of the possible risks against the known benefits supported the continued use of the vaccine [40]. It was later shown that such cases can be due to Dravet syndrome, a rare genetic disorder that may initially present as a febrile seizure following immunisation [41].

A number of national initiatives were undertaken to improve overall vaccine coverage, including the establishment of District Immunisation Co-ordinator posts, the provision of financial incentives for GPs for achieving a target vaccine coverage [36] and the implementation of a national publicity campaign in 1985 [42]. Together with better public awareness of the disease and increasing confidence in the pertussis vaccine, these measures led to a sustained increase in coverage from 1980, which in 1988 reached levels of more than 75% for the first time (Figure 1).

Accelerated schedule

An accelerated 2-, 3- and 4-month primary infant schedule was introduced in England and Wales in June 1990 against a background of improving coverage. UK

studies showed that an accelerated schedule provided satisfactory immunogenicity [43,44] and lower levels of reactogenicity [45] than the previously recommended extended schedule (at 3, 4.5–5 and 8.5–11 months). The decision to recommend a 2-, 3- and 4-month rather than a 2-, 4- and 6-month schedule (as in the US), was based on a number of factors including the recognition that earlier completion of the schedule would afford more rapid protection against pertussis and be likely to improve vaccine coverage. Evidence based on clinic attendance rates suggested that attendance rates began to wane by the time a child reached 7–8 months and thus the move to an accelerated schedule would increase the proportion of infants completing three doses [46]. This Joint Committee on Vaccination and Immunisation recommendation was also in part based on experience in Canada and the US, where schedules starting at the age of 2 months had been associated with a reduction in febrile convulsions [47].

It was later shown that completion of vaccination by the age of 4 months instead of 10 months may have resulted in a four-fold decrease in febrile convulsions attributable to the DTwP vaccine [48]. Despite the change to an accelerated schedule, a booster in the second year of life was not recommended in the UK. Assessment of antibody levels to diphtheria, tetanus and pertussis demonstrated adequate protection with an accelerated schedule (at the age of 3, 4 and 5 months), which persisted to the age of the preschool booster [43].

For the first cohort of children immunised under the accelerated schedule, vaccine coverage at the age of 12 months was identical to that achieved at the age of 18 months on an extended immunisation schedule [49]. This suggested that the immediate impact of the new schedule was to reduce the average age at which children received the third dose. In addition to providing earlier protection, this accelerated schedule was expected to further increase coverage, as the dropout rate for the third dose was lower when scheduled earlier.

In the years following the introduction of the accelerated schedule (in 1990), public confidence in the whole-cell vaccine continued to recover and coverage further increased for all vaccine antigens, including pertussis. Pertussis vaccine coverage by the age of 2 years reached 92% in 1992 and has since exceeded that level (Figure 1). Pertussis notifications in infants continued to fall from a peak of nearly 1,600 notifications in 1990. Peaks of pertussis disease in infants, however, continued to recur at lower levels every three to four years; the highest peak, between 1998 and 2009, was 300 notifications in infants in 2001 [3].

In addition to the overall reduction of disease in infants, the direct impact of the change to an accelerated schedule was apparent in the proportionate distribution of laboratory-confirmed cases in infants.

The proportion of cases in infants aged 6–11 months fell from 50% (1989) to 26% (2008), indicating earlier protection [3]. While there was a concurrent increase in the proportion of cases among infants aged under 3 months (from 17% in 1989 to 50% in 2008), incidence in this age group fell (from 134 per 100,000 in 1989 to 76 per 100,000 in 2008).

Introduction of an acellular booster dose

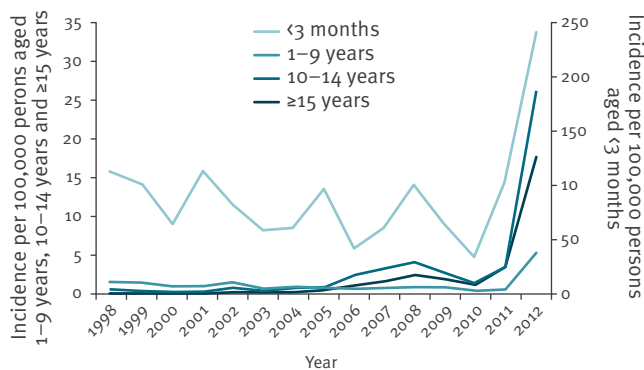
Data from the US in 1993 suggested that waning immunity among adults was becoming an important factor in maintaining disease transmission [50]. The pertussis epidemics in the UK in the 1970s and 1980s would have led to natural boosting in all age groups and it was therefore considered likely that disease transmission at older ages would be delayed for some years [51]. There was concern that early waning of immunity following an accelerated primary course could lead to inadequate immunity in the pre-school years and thus increase transmission to unimmunised young infants from older siblings [51]. However, early data in 1994 following the accelerated schedule did not support this concern [42,52].

Whole-cell vaccine had been shown to have unacceptably high reactogenicity when given as a booster after a whole-cell primary course. Relative efficacy trials of highly purified acellular vaccines and whole-cell vaccines were under way in Sweden by the early 1990s [53,54]. The potential role of acellular pertussis (aP) vaccines for boosting was recognised at that time. The Department of Health in England funded clinical trials to collect immunogenicity and reactogenicity data for the aP vaccine 2-, 3-, 4-month schedule and to generate data on its compatibility with other antigens in the UK schedule [55]. A Swedish multicentre trial of three- and five-component acellular vaccines and the whole-cell vaccine in use in the UK confirmed that the latter was highly effective against mild and severe disease [56].

It had been suggested that the consistent three-to-four-year intervals observed between peak years despite a reduction in incidence indicated that while wP vaccine provided good protection against clinical disease, it was poorly protective against transmission and provided a minimal herd-immunity effect [57]. Any change in disease incidence in infants aged under 3 months must be due to an indirect or herd effect as they are too young to be fully vaccinated. Miller and Gay showed how the epidemic period and the total number of notified cases in infants aged under 3 months up to 1994 fitted with a model that assumed 80% effectiveness against transmission, despite continued three-to-four-yearly cyclical increases in pertussis [58]. This modelling suggested that with sustained high coverage, even without the assumption of waning immunity, disease incidence was likely to increase in older children over 10 years of age and adults.

FIGURE 2

Incidence of laboratory-confirmed pertussis by age group, England and Wales, 1998–2012



Source: Health Protection Agency.

In England and Wales, pertussis notifications from 1990 to 1997 continued to fall, with persistent peak levels of disease every three to four years [59]. While the number of cases in infants aged under 6 months fell overall in this period, the disproportionate pertussis burden in young infants, under-ascertainment of disease burden and the importance of household contacts as a source of infection helped inform the decision to introduce a pre-school pertussis booster. In 2000, based on modelling by the Public Health Laboratory Service [60], the Joint Committee on Vaccination and Immunisation recommended that the most cost-effective introduction of a booster dose would be as an acellular pre-school booster, to indirectly protect infants too young to be immunised [61]. Whole-cell vaccine was not recommended due to its unacceptably high reactogenicity when used as a booster [62].

In November 2001, at a time of high vaccine coverage (94% at 2 years of age for completion of the primary course in England [63]), pertussis was introduced in the pre-school booster as a three- or five-component (DTaP3 or DTaP5) vaccine given with oral polio vaccine (OPV) and measles-mumps-rubella vaccine. This further contributed to the continuing fall in the number of infant cases and an overall reduction in the incidence in children aged under 10 years from 1998 to 2009 (Figure 2) [3]. An initial estimate of the effectiveness of a four-dose schedule was 95.3% (95% CI: 91.9 to 97.2), with cases followed up for up to seven years [3]. A four-dose schedule was estimated to provide an additional 46% (95% CI: -7 to 71) effectiveness compared with three doses [3].

The incorporation of aP vaccines into the primary schedule

The Joint Committee on Vaccination and Immunisation agreed to move from wP to aP vaccines in the primary

schedule when comparable efficacy was demonstrated [64], given the lower rates of systemic and local adverse reactions reported with aP vaccines, particularly in those eligible for the pre-school booster. Another consideration was that the DTwP vaccines contained thiomersal, a mercury-based preservative, while aP vaccines did not. Although there was (and still is) no evidence of harm, this decision was in line with an international aim to reduce children's mercury exposure from avoidable sources. The Joint Committee on Vaccination and Immunisation had previously agreed with the advice from the Committee on the Safety of Medicines to move to thiomersal-free vaccines when effective alternatives were available. The introduction of an aP primary course was also linked to the decision to replace OPV with inactivated polio vaccine (IPV) as soon as progress on global polio eradication reached a point where the risk of importation to the UK had fallen markedly [64]. Consideration of these issues led to the introduction of a combination vaccine including *Haemophilus influenzae b* (Hib) (DTaP-Hib-IPV) into the primary UK schedule from September 2004, replacing the previous DTwP-Hib and OPV vaccines [65].

High primary coverage continued after the introduction of the DTaP-Hib-IPV vaccine (Figure 1) and, from July to September 2011, DTaP-Hib-IPV coverage by the age of 1 year in England was 94.4% and uptake for the pre-school DTaP-IPV vaccine was 85.4% by the age of 5 years [66]. The change in vaccine occurred during the observed fall in incidence in children aged under 10 years between 1998 and 2009 [3]. While notifications and laboratory-confirmed cases in those over 10 years increased over the same period (between 1998 and 2009), the incidence of hospitalised cases remained stable [3]. These increases in teenagers and adults were thought to have been influenced by the introduction of routine serology testing, leading to improved ascertainment in these age groups [3].

In 2011, the overall incidence of pertussis reached a similar level to that in 2008, the previous peak year [67]. The number of confirmed cases in people aged 15 years or older, however, exceeded expected levels and was nearly 50% higher in 2011 than that in 2008 (incidence of 1.6 per 100,000 vs 1.1 per 100,000) [67]. While levels in younger age groups (including infants aged under 3 months) were also high, they were in line with anticipated cyclical increases (Figure 2). This rise in activity continued into the first half of 2012, with increases extending to young infants less than 3 months of age and led to a national outbreak being declared in April 2012 and the introduction of a temporary vaccination programme for pregnant women in October 2012 as an outbreak response measure [68,69]. Pertussis activity peaked in October 2012 and subsequently declined during 2013 across all age groups, in line with normal seasonal trends [70]. Although there has been a substantial fall in the number of cases among infants less than 3 months of age, this outbreak response measure remains in place while high levels of pertussis persist

in those over 15 years of age and we approach the period between July to October where pertussis incidence is typically highest [70].

Discussion

The introduction of routine pertussis immunisation has had a marked impact on the burden of pertussis in England and Wales. The change to an accelerated schedule and sustained high vaccine coverage further reduced pertussis incidence. Since the inclusion of pertussis vaccine in the pre-school booster, there has been a continued reduction in pertussis incidence in children aged 3 months to 9 years. Pertussis incidence is still highest in infants aged under 3 months who cannot be fully protected by immunisation and who are most likely to suffer serious complications. The key aim of the pertussis immunisation programme is to protect these vulnerable infants.

Since the routine availability of serology testing from 2002, the increase in the reported incidence in people aged 10 years and above has been attributed to improved case ascertainment. The recent disproportionate increase in older age groups, however, appears to be a true rise, particularly in those aged 15–40 years. This follows similar increases seen in other countries including Australia, the US and the Netherlands [4,71-73] and is in line with the predictions of Miller and Gay [58]. Although this increase has arisen after the transition to the routine use of the aP vaccine, the age groups affected largely fall outside the aP cohorts (both booster and routine). Vaccine uptake was 78% in 1971, falling to 31% by 1978 then recovering to 92% by 1992. Cohorts born in this period therefore had a lower likelihood of vaccine protection but a greater chance of natural exposure. As pertussis activity fell to consistently lower levels after 1990, boosting through natural exposure was unlikely in all age groups from 1991 to 2010.

The Strategic Group of Experts in Immunisation review concluded in 2010 that the introduction of an adolescent booster should be based on cost-effectiveness [10]. In those countries where adolescent boosters have been introduced (US and Australia), a decreased pertussis burden in the target population has been reported but the impact on the disease in infants is uncertain. Data from the US [74] do not show indirect benefits but the Australian data suggested some benefit for infants when using a school-based catch-up programme (with a broad age group) and a routine programme targeting adolescents aged 12 years and older [75]. A Joint Committee on Vaccination and Immunisation review of the UK adolescent programme is ongoing and inclusion of the pertussis vaccine is being considered. Given the level of under-ascertainment from routine surveillance, accurate estimates of pertussis incidence and burden in adolescents will be essential to inform future cost-effectiveness analyses.

In response to the ongoing national outbreak and the high rates of disease in infants, a temporary vaccination programme for pregnant women was launched in the UK on 1 October 2012 [68,69]. This outbreak response measure was introduced following a review by the Joint Committee on Vaccination and Immunisation of potential vaccine strategies to optimise protection for young infants before their primary course can be delivered [76]. Assessment of the impact and effectiveness of this strategy are current.

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CDC publishes report on antibiotic resistance threats in the United States for the first time

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To raise awareness of the burden and threats posed by antibiotic resistance and to encourage immediate action, the United States (US) Centers for Disease Control and Prevention (CDC) released its first ever report on antibiotic resistance threats in the US – *Antibiotic resistance threats in the United States, 2013* [1].

Antimicrobial resistance represents a serious threat to public health and patient safety and is a worldwide problem. This report is aimed at a wide audience including health professionals and policy makers, as well as the general public.

Section 1 of the report describes the context and provides an overview of antimicrobial resistance in the US. Section 2 describes the four core actions to fight antibiotic resistance: preventing infections and the spread of resistance, tracking resistance patterns, antibiotic stewardship (improving prescribing and improving use) and developing new antibiotics and diagnostic

tests. Section 3 provides a summary for each resistant bacterium considered as a threat for the US and further describes antibiotic resistance, the burden of disease and actions to fight the spread of resistance.

The European Centre for Disease Prevention and Control (ECDC) published a comment on the ECDC website with background information on the situation in Europe and links to relevant publications [2].

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WHO Best practices in prevention, control and care for drug-resistant tuberculosis now published

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The World Health Organization (WHO) Regional Office for Europe published *Best practices in prevention, control and care for drug-resistant tuberculosis* [1] intended as a resource for the continued implementation of the *Consolidated Action Plan to prevent, control and care for multidrug- and extensively drug-resistant tuberculosis in the WHO European Region, 2011–2015* [2].

In order to improve the transfer of knowledge and experiences between countries, and help in improving the health system approach, the WHO Regional Office for Europe launched in May 2013 an initiative to collect examples of best practices in multidrug- and extensively drug-resistant TB (M/XDR-TB) prevention, control and care in the Region. Submission of best practices was open to all stakeholders (ministries of health, national TB control programmes, partners and nongovernmental organisations working to combat tuberculosis and M/XDR-TB in the Region), and examples were collected from May to August 2013.

In total, 82 best practices were submitted from 30 countries. All practices, for which there was enough information (76 practices), were evaluated by an expert committee and the selected best practices were compiled to form this compendium. Best practices are still collected after publication of this compendium, and shared on an online platform linked to the WHO Regional Office for Europe website (<http://www.euro.who.int>).

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