Impact factor 4.659

Eurosurveillance

Europe's journal on infectious disease epidemiology, prevention and control

Vol. 19 | Weekly issue 35 | 04 September 2014

Rapid communications

Research articles

ecdc EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

www.eurosurveillance.org

High incidence of *Plasmodium vivax* malaria in newly arrived Eritrean refugees in Sweden since May 2014

K Sondén[1], E Castro[2], L Trönnberg[2], C Stenström[3], A Tegnell[2], A Färnert (anna.farnert@ki.se)[1,4]
1. Infectious Diseases Unit, Department of Medicine Solna, Karolinska Institutet, Solna, Sweden
2. The Public Health Agency of Sweden, Stockholm, Sweden
3. Department of Clinical Microbiology, Karolinska University Hospital, Solna, Sweden
4. Department of Infectious Diseases, Karolinska University Hospital, Solna, Sweden

Citation style for this article:
Sondén K, Castro E, Trönnberg L, Stenström C, Tegnell A, Färnert A. High incidence of Plasmodium vivax malaria in newly arrived Eritrean refugees in Sweden since May 2014. Euro Surveill. 2014;19(35):pii=20890. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20890

Article submitted on 15 August 2014 / published on 04 September 2014

Since May 2014, an increase in *Plasmodium vivax* malaria has been observed in Sweden. As of 31 August 2014, 105 malaria cases have been reported in newly arrived Eritrean refugees, 84 of them *P. vivax*. The patients were mainly young men and reported migration through Ethiopia and/or Sudan. Severe anaemia and long symptom duration reflect inadequate healthcare during migration. Countries currently hosting Eritrean refugees need to consider *P. vivax* malaria in this group of migrants.

In Sweden, *Plasmodium vivax* accounts for 10 to 15% of the yearly ca 100 imported cases of malaria [1]. We report a dramatic increase in *P. vivax* cases among newly arrived Eritrean refugees, currently the third largest group of refugees in Sweden [2].

Epidemiological hospital-based investigation

P. vivax was diagnosed in seven newly arrived Eritrean refugees seeking care at Karolinska University Hospital in Stockholm in early June 2014. Information about this cluster and an inquiry about additional *P. vivax* cases were sent to the 28 infectious disease departments in Sweden. By 8 August 2014, physicians from 15 hospitals reported to have diagnosed *P. vivax* in 43 newly arrived Eritrean refugees since May 2014. No cases had been reported before May 2014. The patients were predominantly male (32/43) and aged 15 to 34 years (median 21) with 30% minors aged 15 to 17 years. All patients reported travelling from Eritrea through Ethiopia and/or Sudan to Libya and Italy before coming to Sweden between April and June 2014. Haemoglobin levels ranged between 40 and 136 g/L (median: 110 g/L); four patients had haemoglobin levels lower than 70 g/L and received blood transfusions. No other severe symptoms or deaths were reported. The diagnosis was established by conventional microscopy, and the patients were treated with chloroquine, except four patients who received artemether-lumefantrine (Riamet) due to initial uncertain species typing. All patients were prescribed relapse-preventing treatment with a 14-day course of primaquine, after excluding glucose-6-phosphate dehydrogenase deficiency.

National surveillance data

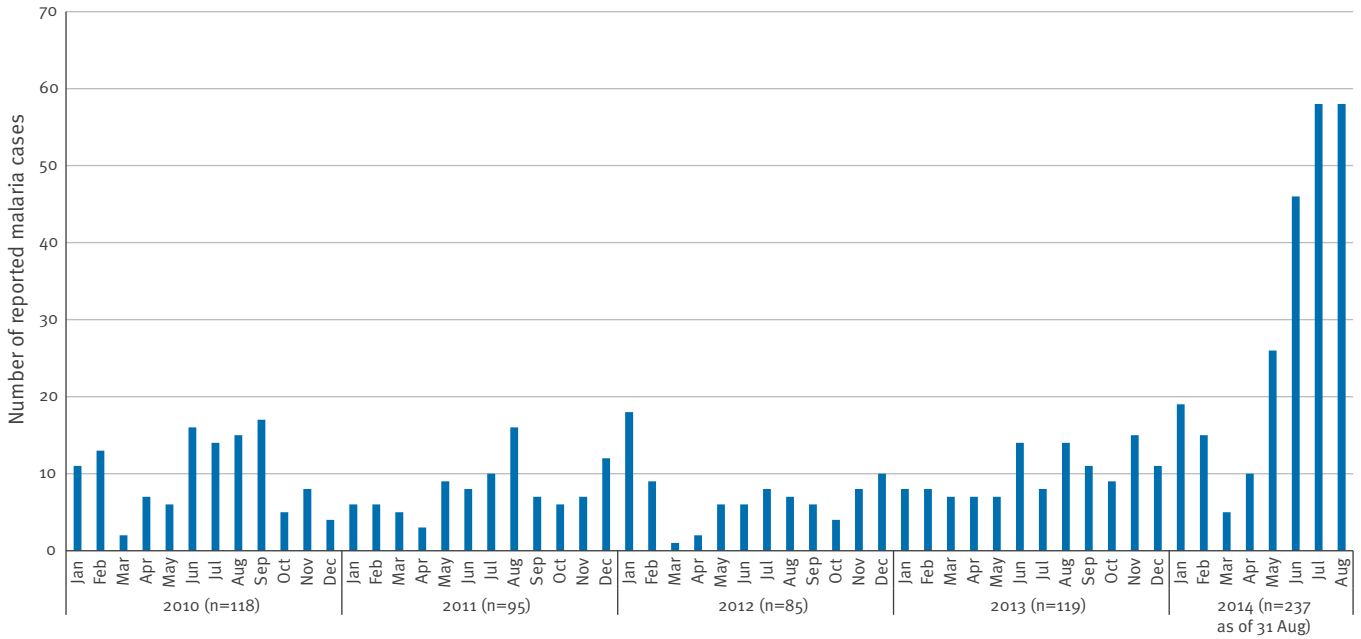
A marked increase in the number of notified malaria cases was also observed at the Public Health Agency of Sweden. This notification is based on mandatory reporting from physicians and diagnostic laboratories according to the Communicable Disease Act (Figure 1).

Until 31 August, 237 cases of malaria have been reported in Sweden in 2014 (102 *P. vivax*, 77 *P. falciparum*, 12 *P. ovale*, two *P. malariae*, 44 unspecified species, and among those three mixed *P. vivax* and *P. falciparum* infections). According to the report from the notifying physician, 115 of them had been infected in Eritrea, Ethiopia and/or Sudan (72 *P. vivax*, seven *P. falciparum*, four *P. ovale*, 32 unspecified species and among those one mixed infection with *P. vivax* and *P. falciparum*). This corresponds to 9.0 *P. vivax* cases per month compared with 0.08 to 0.58 cases per month (between one and seven *P. vivax* cases annually) infected in those countries in the period 2010 to 2013 (Figure 2). There was no concomitant increase in *P. falciparum* cases.

By matching hospital and surveillance data, a total of 105 cases were confirmed to be Eritrean refugees (84 infected with *P. vivax*, five with *P. falciparum*, three with *P. ovale* and 13 with unspecified species). Some cases did not have the country of infection specified in the surveillance system, but were identified as Eritrean refugees in the complementary data provided by clinicians. Details on travel history, other than probable country, are usually not reported to the national surveillance system. Additional cases may therefore have occurred in this group, as well as among the cases where country of infection has not yet been reported.

Figure 1

Number of malaria cases reported to the Public Health Agency, Sweden, 2010–14 (n=654)



Migration and estimated incidence

According to the Swedish Migration Board [2], the annual number of Eritreans seeking asylum was 1,647 in 2011, 2356 in 2012, 4,844 in 2013, and 4,317 (708 minors) in 2014 until June, corresponding to an 1.8-fold increase in applications from 2013 to 2014. The estimated *P. vivax* incidence rate in 2014 was 19.5 per 1,000 Eritrean asylum seekers, and 38.2 per 1,000 Eritrean asylum seekers under 18 years of age. Detailed patient data were not available for 2012 and 2013; a crude estimate was therefore based on all *P. vivax* cases infected in Eritrea, Ethiopia and Sudan, generating an incidence rate of 1.2 in 2012 and 1.4 in 2013.

Discussion

This increase in *P. vivax* malaria cases clustered in newly arrived Eritrean refugees is the largest increase malaria cases diagnosed in Sweden since the reporting to the Swedish Public Health Agency was computerised in 1986. In Sweden, as in other European countries, *P. vivax* is predominantly diagnosed in travellers who have visited Asia or Oceania [3].

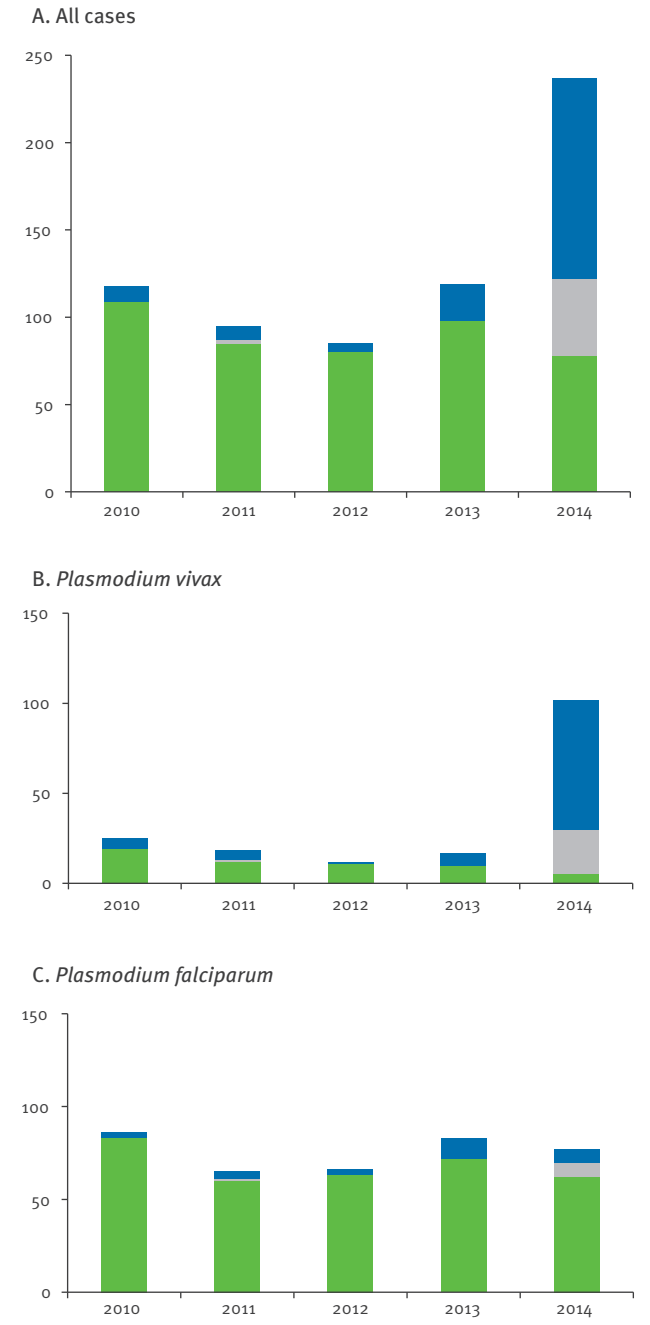
Although the number of asylum applications from Eritreans has increased in Sweden over the last years [2], the estimated *P. vivax* incidence was markedly higher in Eritreans who arrived in the past four months. The patients reported travelling through Ethiopia and/or Sudan. We have not found any reports regarding ongoing outbreaks or markedly increased incidence of *P. vivax* malaria in those countries or in specific refugee camps over the past months. Nonetheless, our data suggest a high transmission of *P. vivax* along the route of migration of Eritrean refugees.

Also other European countries have observed *P. vivax* malaria in Eritrean refugees. In Oslo, Norway, 10 cases were reported to TropNetEurope in June 2014 [M. Jensenius, TropNet, personal communication, June 2014]. The Netherlands has also noted an increase in *P. vivax* cases in Eritreans, coinciding with an increased number of immigrants from that area [M. van der Sande National Institute for Public Health and the Environment, personal communication, July 2014]. A previous cluster of *P. vivax* in Eritrean refugees travelling through Sudan was described in Israel 2010 [4,5].

P. vivax is endemic in more than 90 countries [6], with the highest risk in Central Asia [7]. In sub-Saharan Africa, the parasite is endemic mainly on the Horn of Africa and Madagascar [6], being geographically restricted by absence of Duffy antigens [8]. In Eritrea and Ethiopia, approximately 45% of malaria cases are due to *P. vivax*, compared with 5% in Sudan [6]. Eritrea reports a high rate of anti-malarial interventions and is considered to be on track to reduce malaria cases by more than 75% [6]. Malaria incidence had also decreased in Ethiopia over the last decade but there has been an increase in confirmed malaria cases including *P. vivax* in the past six years [6]. In Sudan, *P. vivax* transmission is limited and restricted to eastern and southern parts of the country [7], the areas where many refugee camps are located [9]. Libya and Italy are considered malaria-free [6]. Several patients described that they and many fellow migrants had symptoms of malaria during the journey; some were treated in Ethiopia and some in Sudan. Although it is difficult to determine the source geographically, it is likely that our patients acquired the infection in Sudan, Ethiopia or Eritrea.

Figure 2*

Number of malaria cases, by origin of infection, reported per year to the Public Health Agency, Sweden 2010–14 (n=654)



Cases from Eritrea, Ethiopia and/or Sudan, reported as probable countries of infection for the newly arrived Eritrean refugees

Cases for whom country of infection has not yet been reported

Cases from other countries (in 2014: Afghanistan, India, Papua New Guinea, Peru and Tanzania)

Data for 2014 are as of 31 August.

Interestingly, although *P. falciparum* is the dominant species in those countries, the increase was specific for *P. vivax*. Geographical hotspots of transmission are likely to be situated along the route of migration, seeing as the *P. vivax* incidence among the refugees was higher than in Eritrea and Ethiopia (in 2012, one and seven per 1,000, respectively [6]). The high incidence, especially among minors, could also suggest a larger malaria-naïve population due to reduced endemicity in Eritrea. This should however also apply for *P. falciparum*, which may have been cleared by previous antimalarial treatments in some of the patients, resulting mainly in *P. vivax* relapses.

Several patients were anaemic and in need of blood transfusions. Malarial anaemia is well described for *P. vivax*, especially after long symptom duration and/or repeated episodes [10].

Conclusions

We report a high number of *P. vivax* malaria diagnosed in Sweden since May 2014. An increased incidence among newly arrived Eritrean refugees, especially in minors, indicates a change in exposure. Importantly, signs of long symptom duration during migration show the need for better healthcare for migrants and highlight the poor conditions that many refugees encounter during their escape. Countries currently hosting Eritrean refugees need to consider *P. vivax* malaria in these migrants.

*Erratum

The legend of Figure 2 was corrected on 5 September 2014. Blue and green were reversed.

Acknowledgements

We wish to thank all the Infectious diseases clinicians who reported on cases, the Swedish Migration Board for providing migration data; as well as Johan Ursing and Urban Hellgren for valuable comments on the manuscript.
Funding statement: Klara Sondén is supported by Karolinska Institutet and has a research/clinical internship at Karolinska University Hospital.

Conflict of interest

None declared.

Author contributions

Conceived the idea of the report: AF, AT, KS. Extracted and analysed data: LT, EC, AT, KS, CS, AF. Wrote the paper: KS, AT, AF. All authors have seen and approved the final manuscript.

References

1. Sjukdomsstatistik: Malaria. [Disease statistics: malaria]. Solna: Folkhälsomyndigheten. [Accessed 8 Aug 2014]. Swedish. Available from: http://www.folkhalsomyndigheten.se/amnesomraden/statistik-och-undersokningar/sjukdomsstatistik/malaria/?t=c
2. Applications for asylum received 1984-2013. . Migrationsverket. [Accessed 8 Aug 2014]. Available from: http://www.migrationsverket.se/download/18.36084ac214622cf6599137e/1403180720423/Tab+2+-+Application+for+asylum+received+1984-2013.pdf
3. Mühlberger N, Jelinek T, Gascon J, Probst M, Zoller T, Schunk M, et al. Epidemiology and clinical features of vivax malaria imported to Europe: sentinel surveillance data from TropNetEurop. Malar J. 2004;3:5. http://dx.doi.org/10.1186/1475-2875-3-5
4. Kopel E, Schwartz E, Amitai Z, Volovik I. Relapsing vivax malaria cluster in Eritrean refugees, Israel, June 2010. Euro Surveill. 2010;15(26):pii=19601.
5. Saidel-Odes L, Riesenberg K, Schlaeffer F, Smolyakov R, Kafka M, Borer A. Eritrean and Sudanese migrants presenting with malaria in Israel. Travel Med Infect Dis. 2011;9(6):303-5. http://dx.doi.org/10.1016/j.tmaid.2011.09.003
6. World Health Organization (WHO). World Malaria Report 2013. Geneva: WHO;2013. Available from: http://www.who.int/malaria/publications/world_malaria_report_2013/en/.
7. Gething PW, Elyazar IR, Moyes CL, Smith DL, Battle KE, Guerra CA, et al. A long neglected world malaria map: Plasmodium vivax endemicity in 2010. PLoS Negl Trop Dis. 2012;6(9):e1814.
8. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to Plasmodium vivax in blacks. The Duffy-blood-group genotype, FyFy. N Engl J Med. 1976;295(6):302-4. http://dx.doi.org/10.1056/NEJM197608052950602
9. United Nations High Commissioner for Refugees (UNHCR). Eritrea. 2014 UNHCR regional operations profile - East and Horn of Africa. Geneva: UNHCR. [Accessed 8 Aug 2014]. Available from: http://www.unhcr.org/pages/49e4838e6.html
10. Douglas NM, Anstey NM, Buffet PA, Poespoprodjo JR, Yeo TW, White NJ, et al. The anaemia of Plasmodium vivax malaria. Malar J. 2012;11:135. http://dx.doi.org/10.1186/1475-2875-11-135

Measles virus spread initiated at international mass gatherings in Europe, 2011

S Santibanez (SantibanezS@rki.de)[1], K Prosenc[2], D Lohr[3], G Pfaff[3], O Jordan Markocic[4], A Mankertz[1]
1. National Reference Centre Measles, Mumps, Rubella and Regional Reference Laboratory WHO EURO, Division of Measles, Mumps, Rubella, and viruses affecting immunocompromised patients, Robert Koch Institute, Berlin, Germany
2. Laboratory for Virology, National Institute of Public Health of Slovenia, Ljubljana, Slovenia
3. Baden-Wuerttemberg State Health Office, District of Stuttgart Government, Stuttgart, Germany
4. Regional Institute of Public Health of Ljubljana, Slovenia

Citation style for this article:
Santibanez S, Prosenc K, Lohr D, Pfaff G, Jordan O, Mankertz A. Measles virus spread initiated at international mass gatherings in Europe, 2011. Euro Surveill. 2014;19(35):pii=20891. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20891

Article submitted on 22 March 2013 / published on 04 September 2014

Three parallel transmission chains of measles virus (MV) variant 'D8-Villupuram' (D8-V) originated from two coinciding international mass gathering (MG) events in Rimini, Italy, in June 2011. MV D8-V was independently introduced into Germany by two unvaccinated persons, and into Slovenia by one unvaccinated person who had attended these events. Secondary spread of D8-V was restricted to two generations of transmission in Slovenia as well as in Germany where the virus was further disseminated at another MG. Serological and epidemiological investigation of the D8-V-associated German and Slovenian cases revealed different antibody responses and age distributions. Primary infected young persons between 11 and 27 years-old were affected in Germany, whereas the group of Slovenian cases comprised adults aged from 28 to 47 years and a high proportion (9/14; 64%) of patients with secondary vaccine failure (SVF). Our study demonstrates that monitoring of MV transmission chains in an international context and adequate serological investigation of cases with remote vaccination can contribute to identify susceptibility gaps.

Introduction

The World Health Organization (WHO) European Region (EUR) has adopted the goal of eliminating endemic measles by 2015. In this region, a vaccine coverage of ≥95% with two doses of a measles containing vaccine (MCV) must be achieved and maintained in order to terminate endemic transmission of measles virus (MV) and reach a low annual incidence (‹1 case per 1 million population) [1]. However, the current measles situation in EUR is characterised by considerable differences in vaccine coverage and incidence across and within countries. Only a few countries report absence or sporadic occurrence of measles with a low annual incidence while others observe resurgence of measles after a long period of low incidence (e.g. Slovenia) or are still experiencing outbreaks with hundreds or thousands of cases (e.g. Germany and Italy) [2]. In 2013, the majority of the EUR countries have reported an annual incidence of ›1 case per one million population (http://data.euro.who.int/cisid/?TabID=335279, data as 02 April 2014) indicating that the elimination target is not yet met. This situation implies the urgent need to uncover and analyse chains of MV transmission with the objective to identify vulnerable groups in the European population.

Measles is an aerosol-borne and highly contagious viral disease characterised by fever and rash. The incubation period ranges from seven to 21 days from exposure to onset of fever and the rash usually appears about 14 days after exposure [3]. A person infected with MV is highly infectious a few days before the rash appears. Contagious but not yet symptomatic individuals can deliver MV to others and may not be recognised as a source of infection. At mass gatherings (MGs), the high number of participants in a crowded setting can further increase the risk of MV transmission [4]. MV long-distance spread has repeatedly originated at international MGs by participants travelling back to their home country [5-7] and, as in our study, has even been transmitted from one MG to the next. MGs therefore represent a test for countries approaching measles elimination. The present study investigates MV transmission chains that were initiated at two coinciding international MGs held in Rimini, Italy, in June 2011. We demonstrate that an adequate serological and molecular-epidemiological characterisation of cases linked to MGs may be helpful in tracing international MV transmission pathways and identifying unprotected population groups.

Methods

Clinical case definition

Measles cases that met the case definition of clinical measles used by the WHO were included into this study: 'Measles is an illness characterized by generalized maculopapular rash lasting 3 or more days with a temperature of 38.3°C (101°F) or higher, and cough, coryza, or conjunctivitis' [8].

Collection of clinical samples and case-based data

Measles is notifiable in Germany since 2001 [9] and in Slovenia since 1948 [10]. Laboratory confirmation of notified cases of suspected measles is performed by the WHO measles/rubella national reference laboratories (NRLs) of Germany and Slovenia. The NRLs send sample collection devices with instructions and a laboratory submission form to local public health authorities, paediatricians and general practitioners as well as hospitals. The form collects the patients' identifier, date of birth, sex and age, and asks whether the case definition is met. Furthermore, data on specimen collection, onset of rash, complications, immunisation status, attendance of community institutions (e.g. kindergartens, schools or hospitals), travel anamnesis, attendance at MGs and whether an epidemiological link to another case is known are requested by the form. The form is filled out by the ambulatory physician or by a medical doctor of the local public health institution. Additional epidemiological information is provided by the local public health institution via electronic mail or telephone. The case-based data are deposited in the databases of the NRLs.

Collection of data on immunisation status

Data from the immunisation certificate were entered into the laboratory request form by the treating physician (number of doses, dates of vaccination). If the immunisation certificate was not available, the vaccination status was either classified as 'unknown' or as 'vaccinated in accordance to the schedule of mandatory measles vaccination' (Slovenia). In Slovenia, a MCV was offered first to children born in 1960 and 1961 and became mandatory for unprotected children born since 1962 at school entry. Children born from 1968 onwards have been vaccinated at eight months of age and children born since 1969 received a second dose at the age of five years. Since then, mandatory vaccination with two doses of a MCV has been applied. The combined measles, mumps, rubella vaccine (MMR) is given to children born since 1989. In other republics of the former Yugoslavia, introduction of routine measles vaccination started later than in Slovenia. In the former German Democratic Republic, vaccination with a MCV was mandatory since 1970 with one dose and since 1986 with two doses. In the former Federal Republic of Germany, one dose was recommended since 1974. In 1991, a nationwide two-dose MCV schedule with voluntary application was adopted in Germany; since 2001, the first dose has been recommended at 11 to 14 months and the second dose at 15 to 23 months [11].

Laboratory confirmation of suspected cases

Suspected clinical cases of measles were laboratory confirmed by detection of MV RNA and/or MV-specific IgM antibodies.

Serological investigation and classification of cases

MV-specific IgM and IgG antibodies in serum were determined by enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN AG, Luebeck, Germany). To discriminate between primary infection and secondary infection (reinfection), the IgG avidity index (AI) was determined in cases with MV RNA detection, by IgG Avidity ELISA (EUROIMMUN AG, Luebeck, Germany). A low AI (‹20%) indicates a primary infection and a high AI (≥60%) a reinfection. A reinfection in a patient with a remote history of vaccination (›6 weeks) is referred to as secondary vaccine failure (SVF). For cases exhibiting intermediate AI values (≥20% and ‹60%), case classification considered the interval between onset of symptoms and collection of serum.

Detection and genetic identification of measles virus

Genetic identification of MV detected in throat swab (TS) samples was performed by sequencing the 450 nt coding for the carboxy-terminal 150 amino acids of the nucleoprotein (N-450) and phylogenetic analysis [12,13] as recommended by the WHO [14]. Representative MV sequence data were submitted to the WHO Measles nucleotide surveillance (MeaNS) database [15] and to GenBank.

Criteria for case assignment

A measles case is considered infectious from five days before until four days after onset of rash. Successive measles cases are epidemiologically linked to each other when a subsequent case was in contact with an infectious case seven to 18 days before the onset of rash [16]. A case was assigned to an identified transmission chain if it met one of the following criteria:

(i) Criterion 1: Case is infected with MV exhibiting sequence identity to that of the index case (MV variant D8-Villupuram 'D8-V') and the case is epidemiologically linked (directly or via a chain of successive cases) to the index case.
(ii) Criterion 2: Case without MV genotype information that has been laboratory-confirmed or not laboratory investigated is epidemiologically linked directly to the index case or a case that meets criterion 1.

Results

Pathways of measles virus transmission

The 16th Italia Super Cup, an international youth football tournament, was held close to Rimini, Italy, between 2 and 5 June 2011. Two German participants showed measles symptoms after returning to their place of residence in town A, German federal state of Baden-Wuerttemberg, on 15 and 17 June (week 24). The time span until onset of disease suggests that both cases may have contracted the virus during the football tournament (Table, Figure 1). The first case (16 year-old, unvaccinated) spread the infection to two unvaccinated siblings. The second case (18 years-old, unvaccinated)

Table 1a

Immunisation status, clinical data, demographic characteristics and laboratory findings of measles cases epidemiologically linked to mass gathering events in Rimini, Italy, June 2011 (n=28)

Case number	Town[a]	Sex	Age in years	Immunisation status (MCV)	Onset of illness date (week number)	Sampling date (week number)	MV-specific antibodies			Laboratory confirmation and case classification	MV genotype and variant	GenBank accession number and WHO name
							IgM	IgG	IgG avidity index			
MV transmission chain, Rimini-Baden-Wuerttemberg-1												
1.1 (index case)	A	M	16	Unvaccinated	15/06/11 (24)	21/06/11 (25)	+	+	12%	Yes, primary infection	D8-V	KJ437153 MVs/Sigmaringen.DEU/25.11
1.2	A	M	19	Unvaccinated	27/06/11 (26)	–	ND	ND	ND	NA	ND	–
1.3	A	F	14	Unvaccinated	27/06/11 (26)	–	ND	ND	ND	NA	ND	–
MV transmission chain, Rimini-Baden-Wuerttemberg-2												
2.1 (index case)	A	M	18	Unknown	17/06/11 (24)	21/06/11 (25)	+	+	13%	Yes, primary infection	D8-V	–
2.2	B	F	18	Unvaccinated	25/06/11 (25)	NA	+[b]	ND	ND	Yes, not classified	ND	–
2.3	A	F	21	Unvaccinated	26/06/11 (25)	NA	+[b]	ND	ND	Yes, not classified	ND	–
2.4	C	M	27	Unvaccinated	26/06/11 (25)	NA	+[b]	+[b]	ND	Yes, not classified	ND	–
2.5	D	M	23	Unvaccinated	27/06/11 (26)	NA	+[b]	ND	ND	Yes, not classified	ND	–
2.6	E	M	20	Unknown	27/06/11 (26)	07/07/11 (27)	+[b]	ND	ND	Yes, not classified	D8-V	KJ437154 MVs/Mannheim.DEU/27.11
2.7	A	M	20	Unvaccinated	28/06/11 (26)	05/07/11 (27)	+	+	5%	Yes, primary infection	D8-V	–
2.8	F	M	24	Unvaccinated	30/06/11 (26)	06/07/11 (27)	+	+	5%	Yes, primary infection	D8-V	KJ437155 MVs/Heidelberg.DEU/27.11
2.9	A	F	15	Unvaccinated	04/07/11 (27)	–	ND	ND	ND	NA	ND	–
2.10	G	M	11	Unvaccinated	08/07/11 (27)	11/07/11 (28)	-	-	ND	Yes, primary infection	D8-V	KJ437156 MVs/Radolfzell.DEU/28.11
MV transmission chain, Rimini-Slovenia												
3.1 (index case)	H	F	34	Unvaccinated	13/06/11 (24)	19/06/11 (24)	+	-/+	ND	Yes, primary infection	D8-V	KJ411829 MVs/Ljubljana.SVN/24.11
3.2	I	M	34	Unvaccinated	28/06/11 (26)	01/07/11 (26)	-	-	ND	Yes, primary infection	D8-V	KJ411827 MVs/Celje.SVN/26.11
3.3	J	M	33	Unvaccinated	30/06/11 (26)	03/07/11 (26)	+	-	ND	Yes, primary infection	D8-V	KJ411828 MVs/Kranj.SVN/26.11
3.4	H	M	30	Vaccinated[c] in 1981 and 1987	01/07/11 (26)	05/07/11 (27)	-	+	83%	Yes, SVF	D8-V	–

+: positive; -: negative; -/+: borderline.

MCV: measles-containing vaccine; MV: measles virus; NA: not available; ND: not determined; SVF: secondary vaccine failure; WHO: World Health Organization.

[a] In Germany towns B, C, D, E, F, G are all located at a distance of town A varying between 35 km and 165 km. In Slovenia, towns I, J, K are all located within a distance of town H varying between 24 km and 62 km.

[b] Investigation performed by external laboratory.

[c] Immunisation record not available, vaccination is considered according to the schedule of mandatory measles vaccination used in Slovenia for children born 1962 onwards.

[d] These cases probably belong to the MV transmission chain 'Rimini-Slovenia'.

Table 1b

Immunisation status, clinical data, demographic characteristics and laboratory findings of measles cases epidemiologically linked to mass gathering events in Rimini, Italy, June 2011 (n=28)

Case number	Town[a]	Sex	Age in years	Immunisation status (MCV)	Onset of illness date (week number)	Sampling date (week number)	MV-specific antibodies			Laboratory confirmation and case classification	MV genotype and variant	GenBank accession number and WHO name
							IgM	IgG	IgG avidity index			
MV transmission chain, Rimini-Slovenia												
3.5	H	F	46	Vaccinated[c] in 1970	01/07/11 (26)	04/07/11 (27)	-	+	89%	Yes, SVF	MV RNA detected, genotype ND	–
3.6	H	F	28	Vaccinated in 1984	29/06/11 (26)	01/07/11 (26)	ND	ND	ND	Yes, VF not classifiable	D8-V	–
3.7	H	F	42	Vaccinated[c] in 1969	02/07/11 (26)	04/07/11 (27)	+	+	78%	Yes, SVF	D8-V	–
3.8	K	M	36	Vaccinated[c] in 1975 and 1981	02/07/11 (26)	05/07/11 (27)	-	+	74%	Yes, SVF	D8-V	KJ411831 MVs/Novo Mesto.SVN/27.11
3.9	H	F	47	Unvaccinated	02/07/11 (26)	06/07/11 (27)	+	-/+	ND	Yes, primary infection	D8-V	–
3.10	H	M	35	Vaccinated[c] in 1977 and 1983	04/07/11 (27)	04/07/11 (w. 27)	-	+	75%	Yes, SVF	MV RNA detected, genotype ND	–
3.11	H	M	33	Vaccinated[c] in 1978 and 1984	NA	04/07/11 (27)	-	+	89%	Yes, SVF	D8-V	–
3.12	H	M	32	Vaccinated in 1979 and 1985 (documented)	NA	05/07/11 (27)	+	+	42%	Yes, probable SVF	D8-V	–
3.13	H	F	33	Vaccinated in 1978 and 1984 (documented)	13/07/11 (28)	15/07/11 (28)	+	+	82%	Yes, SVF	D8-V	–
4[d]	H	F	44	Vaccinated in 1973	29/06/11 (26)	04/07/11 (27)	+	+	69%	Yes, SVF	D8-V	–
5[d]	H	M	35	Unvaccinated	11/07/11 (28)	16/07/11 (28)	+	-	ND	Yes, primary infection	D8-V	KJ411830 MVs/Ljubljana.SVN/28.11

+: positive; -: negative; -/+: borderline.

MCV: measles-containing vaccine; MV: measles virus; NA: not available; ND: not determined; SVF: secondary vaccine failure; WHO: World Health Organization.

[a] In Germany towns B, C, D, E, F, G are all located at a distance of town A varying between 35 km and 165 km. In Slovenia, towns I, J, K are all located within a distance of town H varying between 24 km and 62 km.

[b] Investigation performed by external laboratory.

[c] Immunisation record not available, vaccination is considered according to the schedule of mandatory measles vaccination used in Slovenia for children born 1962 onwards.

[d] These cases probably belong to the MV transmission chain 'Rimini-Slovenia'.

fell ill while he attended a music festival with 50,000 visitors ('Southside Festival') in Neuhausen ob Eck (Baden-Wuerttemberg) between 17 and 19 June, 2011. Seven participants of this festival developed symptoms of measles between 25 and 30 June (weeks 25 and 26). One of them passed the infection on to two relatives who showed measles symptoms on 4 and 8 July (week 27).

The outbreak investigation performed by the public health authorities revealed two transmission chains with a total of 13 epidemiologically linked cases in Baden-Wuerttemberg (Figure 2). The presumed epidemiological link between the Baden-Wuerttemberg cases was confirmed by detecting the identical MV variant (MVi/Villupuram.IND/03.07) in the two index cases, three of nine secondary cases and one of two tertiary cases. This variant was first identified in 2007 in Villupuram, India. Since it is a predominant variant, MVi/Villupuram.IND/03.07 is one of the few 'named strains' in the Measles Nucleotide Surveillance (MeaNS) database (GenBank accession number: FJ765078) and referred to as 'D8-Villupuram' (D8-V).

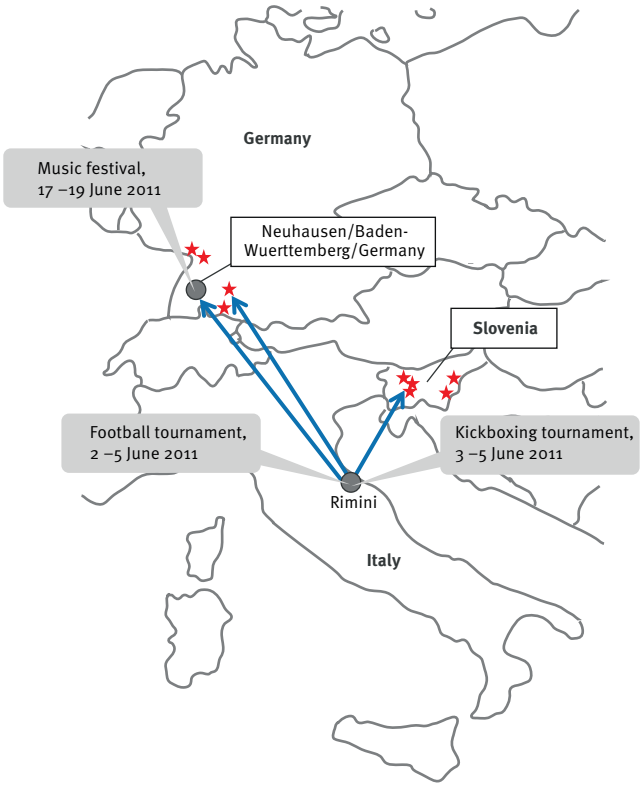
Another international sport event, the World Association of Kickboxing Organizations (WAKO) Bestfighter World Cup Tournament with 2,100 visitors was held in Rimini at the same time (3–5 June 2011). An unvaccinated 34 year-old woman born in the former Yugoslav Republic of Macedonia and residing near town H in Slovenia, had attended the event as a seminar participant. After her return to Slovenia, she developed symptoms of measles on 17 June (week 24); the time line suggests that she may have acquired the infection during her stay in Rimini (Table, Figure 1). Eleven contacts in Slovenia fell ill between 28 June and 4 July (weeks 26 and 27) (Figure 3). Eight of these cases occurred in town H, while three respectively occurred in different towns located at a distance varying between 24 km and 62 km of town H. One of the town H cases infected his spouse who showed rash and fever on 13 July in (week 28). MV variant D8-V was detected in the index case, nine of 11 secondary cases and the only tertiary case, indicating spread of the imported virus in Slovenia. Two D8-V associated cases occurred in town H on 29 June (week 26) and 11 July (week 28), but contacts to the identified D8-V transmission chain have not been recognised (Figure 3).

Measles virus transmissions in healthcare and community institutions in Slovenia

The majority of transmissions of D8-V MV in Slovenia occurred in healthcare and community institutions. When the index case first presented at a community healthcare centre near town H on 15 June, she may have transmitted the virus to a person visiting this centre on the same day, who subsequently was among the secondary cases investigated. The index patient also transmitted the virus to a staff member of the kindergarten which her child frequented. Nine secondary cases were putatively infected in a medical centre in town H on 19 June. Among them were five staff members (3 nurses and 2 facility managers), three patients waiting in the same corridor as the index case and a visitor of the clinic. The index case was not immediately recognised as a measles case and consequently not isolated for several hours.

Figure 1

Long-distance spread and local distribution of cases of measles virus variant D8-Villupuram (D8-V) epidemiologically linked to mass gatherings in Rimini, Italy, June 2011 (n=28)



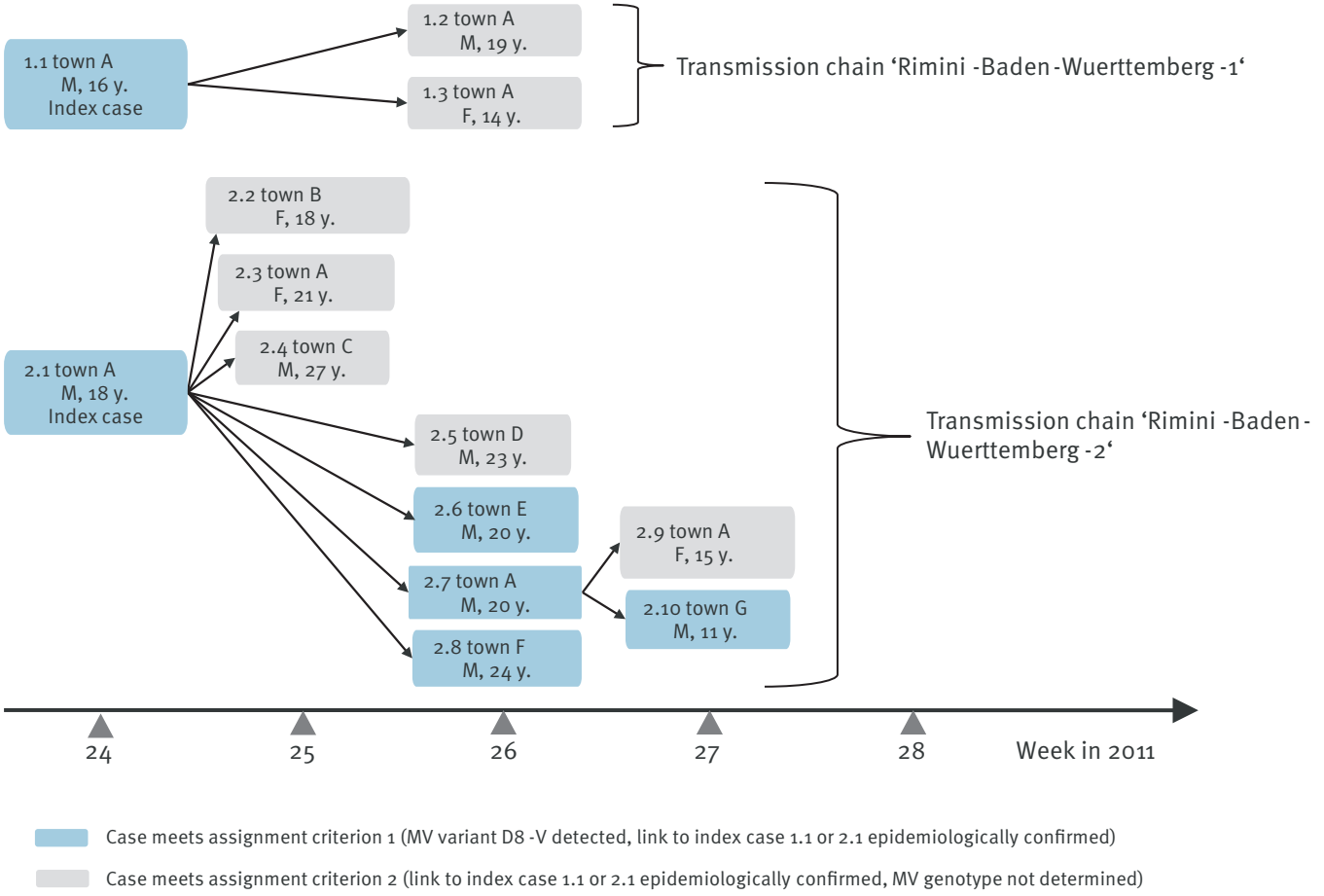
[a] Criterion 1: case is infected with measles virus exhibiting sequence identity to variant D8-V and the case is epidemiologically linked (directly or via a chain of successive cases) to the index case.

Source of identified measles virus

MV variant D8-V has been continuously detected in India from 2007 onwards (MVs/Kalahandi.IND/04.08/2, GenBank accession number: EU812246; MVs/Dimapur.IND/14.09, HM773267; MVi/Kasargod.IND/12.10, HM358877; MVs/Pune.IND/07.11, JQ083634). The virus has been repeatedly introduced into Europe, for example by a German tourist from Baden-Wuerttemberg returning from India in February 2011 (MVs/Tuebingen.DEU/08.11). Immediately before the international MGs in Rimini started, D8-V was detected in several provinces of central and northern Italy (MVs/Perugia.

Figure 2

Chains of transmission of measles virus variant D8-Villupuram (D8-V) identified in Baden-Wuerttemberg, Germany, June–July 2011 (n=13 cases)



F: female; M: male; MV: measles virus; y: years (age of cases is given in years).
The week given for each case refers to the date of onset of illness.

Towns B, C, D, E, F and G were at a distance of town A varying between 35 and 165 km.

ITA/15.11/3, MVs/Gubbio.ITA/16.11, MVs/Monza.ITA/21.11, MVs/LAquila.ITA/22.11, F. Magurano, personal communication, March 2014, MeaNS database). Outside of Italy, D8-V was only sporadically observed at this time (MVs/Pforzheim.DEU/17.11; MVs/London.GBR/18.11/2, MVs/Bristol.GBR/21.11, K.E. Brown, personal communication, March 2014, MeaNS database; MVs/Douai.FRA/22.11, F. Freymuth, J. Dina, personal communication, March 2014, MeaNS database). A D8-V-associated case not linked to the MGs mentioned above was detected in week 16 2011 in Freiburg, Baden-Wuerttemberg (MVs/Freiburg.DEU.26.11), when a child had contact to a case who had acquired the infection in Rome, Italy. In summary, these data indicate that D8-V was a contemporary virus in Italy in spring 2011.

Case classification

MV-serology was used to classify cases with evidence of MV shedding (MV RNA detection positive) either as primary infection or reinfection. Previously vaccinated cases with evidence of reinfection were classified as SVF.

Cases in Germany

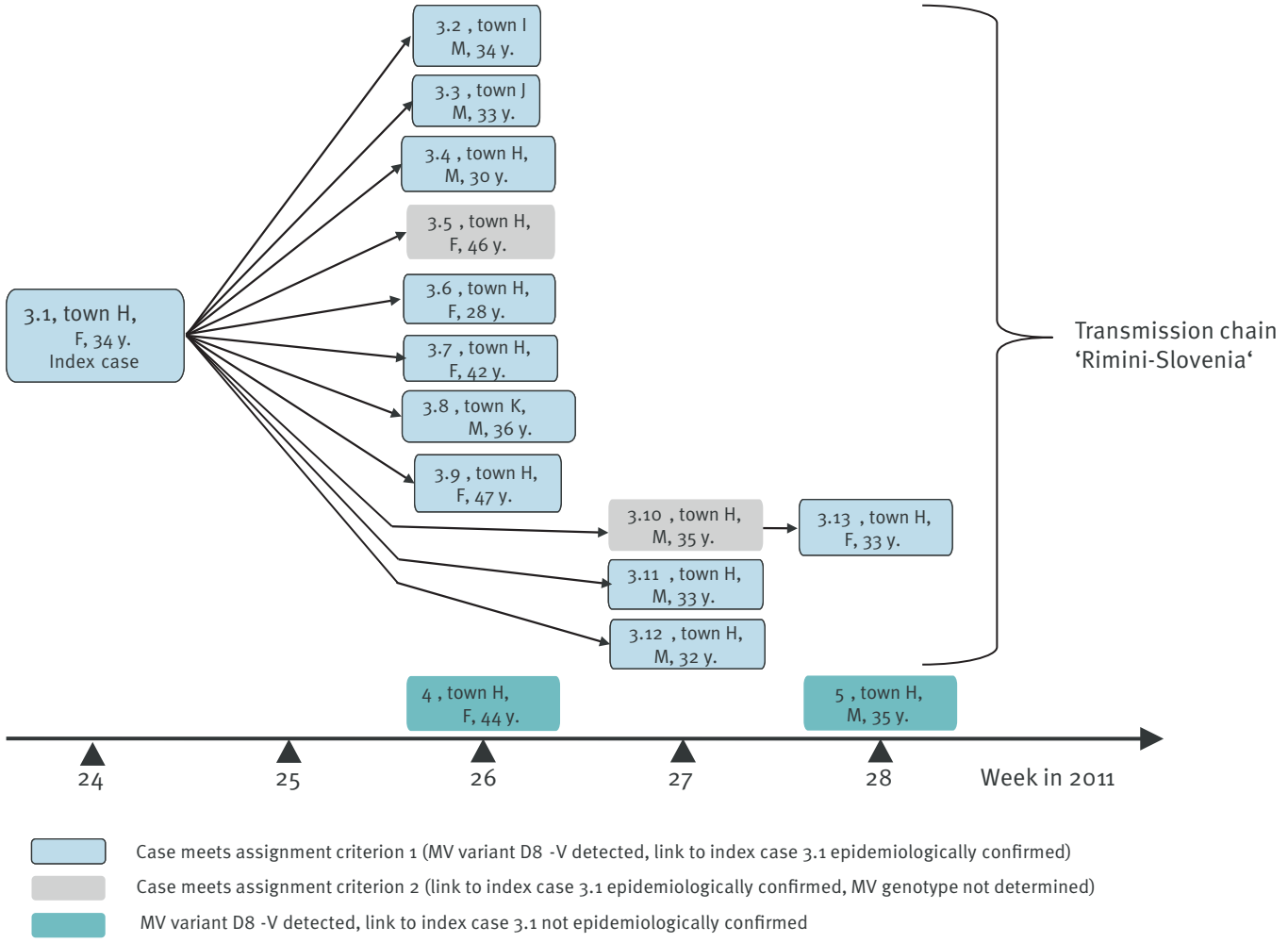
Of the 13 measles cases in Baden-Wuerttemberg, serological test results were obtained for 10 (Table). Of these 10, three unvaccinated cases (cases 1.1, 2.7 and 2.8) and one case with unknown vaccination status (case 2.1) were IgM positive and exhibited a low AI. Another unvaccinated case (case 2.10) was negative for IgM and IgG. These data show primary infection in all (5/5) classified cases in Baden-Wuerttemberg. The remaining five cases were confirmed by a positive IgM in an external laboratory and could not be classified (cases 2.2, 2.3, 2.4, 2.5 and 2.6).

Cases in Slovenia

Fourteen of 15 laboratory-confirmed cases were classified by serology (Table). Of these 14 cases, 12 were confirmed by epidemiological link to belong to the transmission chain 'Rimini-Slovenia' whereas two were

Figure 3

Chain of transmission of measles virus variant D8-Villupuram (D8-V) identified in Slovenia, June–July 2011 (n=15 cases)



M: male, F: female; y: years (age of cases is given in years).

The week given for each case refers to the date of onset of illness or sample collection (cases 3.11 and 3.12).
Towns I, J and K were at a distance of town H varying between 24 km and 62 km.

assigned putatively by molecular evidence (cases 4 and 5). Four of the five unvaccinated cases were IgM positive with negative or borderline IgG values (cases 3.1, 3.9, 3.3 and 5) and one case was negative for IgM and IgG (case 3.2); indicating a primary infection. One case with two documented doses of a MCV (case 3.12) exhibited a positive IgM and an intermediate AI and was classified as a probable SVF. A second case with two documented vaccine doses (case 3.13) had a positive IgM and a high AI indicating a SVF. Seven cases could not provide documentation of vaccination. According to the Slovenian vaccination schedule, these individuals should have received one dose (cases 3.5, 3.7 and 4) or two doses of a MCV (cases 3.4, 3.8, 3.10 and 3.11). All had a high AI and were therefore also classified as SVF. Among them were two IgM positive (cases 3.7 and 4) and five IgM negative cases (cases 3.4, 3.8, 3.5, 3.10 and 3.11). Overall, 5/14 (36%) primary infected cases, 8/14 (57%) cases with a SVF and one case (7%) with a probable SVF have been observed in Slovenia. The SVF cases had received the last vaccination 27 to 42 years previously.

Age distribution

Cases in Germany

The 13 cases assigned to the two transmission chains in Baden-Wuerttemberg were 11 to 27 years-old and included one child (‹14 years-old), three adolescents (›14 and ‹18 years-old) and nine young adults (›18 and ‹28 years-old). Overall the median age of the German cases was 19 years and the arithmetic mean 18.9 years.

Cases in Slovenia

The 13 cases assigned to the transmission chain in Slovenia and the two putatively associated cases were

adults aged from 28 to 47 years. A median of 34 years and an arithmetic mean of 36.1 years were calculated. The cases classified as primary infection (n=5) were 33 to 47 years-old (median: 34 years, arithmetic mean: 36.6 years), and the cases of SVF (n=8) or probable SVF (n=1) were 30 to 46 years-old (median: 35 years, arithmetic mean: 36.8 years); the unclassified case was 28 years-old.

Discussion

A molecular-epidemiological analysis enabled us to trace the spread of MV D8-V in Europe disseminated via three international MGs (Figure 1). Two of the MGs were held in Rimini, Italy, the third was held in Germany. Measles spread in the German MG via a case epidemiologically linked to one of the Italian events. In 2011, indigenous MV transmission and a high measles incidence (85.4 cases per 1 million population) had been documented in Italy where measles is notifiable and vaccination with two doses of a MCV is recommended [17,18]. In comparison, measles incidences in Germany and Slovenia were 19.5 and 10.8 cases per one million population respectively [17]. D8-V was frequently detected in central and northern Italy immediately before the MG in Rimini started (F. Magurano, personal communication, March 2014). This finding suggests that dissemination of MV D8-V to Germany and Slovenia was linked to a source in Italy and stresses the high risk of measles exposure at MGs in a country with high measles incidence.

Two transmission chains in Germany and one in Slovenia were initiated by unvaccinated participants (Figures 2 and 3). Transmission of the imported D8-V in Germany resulted in two chains with a total of 13 identified cases that occurred in the Federal State of Baden-Wuerttemberg. The first German index case initiated only one generation of familial MV transmission (two cases); the second disseminated the virus at a music festival in Baden-Wuerttemberg with 50,000 participants, initiating two generations of virus transmission apparently restricted to Baden-Wuerttemberg. The chain in Slovenia comprised two generations of virus transmission with 13 assigned cases plus two putative cases.

Multiple transmissions of D8-V indicate that pockets of susceptible persons persist in Germany as well as in Slovenia. In Germany, the immunisation coverage increased from 2000 to 2010 for the first dose from 91.1% to 96.4% and for the second dose from 19.4% to 91.5% [19]. In contrast, in Slovenia where vaccination with two doses of a MCV has been mandatory for children born since 1969, >95% coverage for the second dose has been sustained since 1983 [10]. Slovenia had reported absence of measles cases from 2000 to 2009, and a low incidence of one case per one million population in 2010 [10], whereas Germany had continuously experienced an annual incidence of >1 case per one million population [2,20]. The resurgence of measles in Slovenia demonstrates that absence of indigenous transmission over a long period cannot be equated with the absence of pockets of susceptibles in the population. The short circulation period of <6 weeks for the imported D8-V suggests that the proportion of susceptibles in the Slovenian as well as in the German population was not high enough to allow sustained transmission.

Twelve of the 13 German cases were unvaccinated and 11 to 27 years-old, adding evidence to a lack of protection in this age group [6,9,11]. In contrast, older individuals aged 28 to 47 years were affected in Slovenia. Moreover, there was no age difference between the unvaccinated primary infected Slovenian cases (n=5; mean age: 36.6 years, median age: 34 years) and the SVF cases (n=9; mean age: 36.8 years, median age: 35 years). Two previous Slovenian cases associated with other MV genotypes (B3 and D4, data not shown) were of the same age group, indicating that the older age of the Slovenian cases was not particular for the transmission chain of D8-V. Recent outbreaks in the EUR were characterised by lower median ages, e.g. of 11 years for the epidemic in Switzerland between 2006 and 2009 and of 13.5 years in Bulgaria between 2009 and 2011 [13,21]. The unusual high age of the cases in Slovenia may be due to absence of measles for a decade and the resulting shift of the susceptibles to higher age groups.

The proportion of SVF among the Slovenian cases was remarkably high (9/14; 64%). Case reports of SVF, i.e. measles infection in individuals with a previously documented seroconversion after MCV vaccination, are considered rare [22]. However, several reports describe a significant proportion of SVF in populations with sustained high vaccination coverage [23-25] after long absence of MV transmission with the resultant lack of natural boosting, and waning of both the concentration as well as the avidity of anti-measles IgG antibodies [26]. Since the vaccination coverage in Slovenia has been continuously high over a long period, waned immunity may explain the high proportion of SVF. In case of SVF, MV replicates in presence of pre-existing vaccine induced neutralising antibodies. Viral replication and transmission is therefore limited and spread of MV occurs rarely, if at all [27]. The transmission chain in Slovenia showed two successive SVF cases within a family, indicating that a symptomatic SVF case can contribute to MV transmission. Our observation may serve as an incentive to monitor SVF more carefully, since the risk of emerging vaccine-escape variants is enhanced in such a setting due to exposure of virus to vaccine induced neutralising antibodies.

In Slovenia, MV D8-V was transmitted nosocomially in healthcare institutions (six infected healthcare workers, HCWs) or in community facilities. Only one 47 year-old HCW showed a primary infection that could have been prevented by a prior vaccination. This case demonstrates the need of providing evidence of protection by documentation of two doses of a MCV and/

or a positive MV-specific IgG for all HCWs regardless of their year of birth. The five other cases occurred among previously vaccinated HCWs due to SVF. None of the infected HCWs caused a further nosocomial transmission, which might be explained by a reduced viral shedding in case of SVF. Measles among both unvaccinated and vaccinated HCWs has also been reported from recent epidemics in Europe [28,29], but the role of SVF has rarely been investigated [30].

Our study highlights the high risk of contracting MV at international MGs in Europe and shows that MV D8-V has spread from Italy to Germany and Slovenia with subsequent local transmission. The restricted length of the identified local chains to two generations of transmission suggests that the immunisation coverage in the affected regions was high enough to prevent sustained MV transmission. We identified once more unvaccinated adolescents and young adults as a vulnerable group in Germany [31], whereas transmission of D8-V in Slovenia was observed in young and middle aged adults, most of whom were vaccinated (10 cases of 15). The finding of a high proportion of SVF (9/14; 64%) among the Slovenian cases emphasises the necessity of laboratory-based case investigation as well as studies assessing population immunity in countries with long absence of MV circulation like Slovenia. In highly vaccinated populations, suspected measles infection in patients with a remote history of vaccination should be investigated by viral RNA detection, IgM, IgG and IgG avidity testing to uncover vaccine failure. SVF and its contribution to measles transmission should be surveyed closely. Participants of MG should check their vaccination status and those who cannot provide evidence of protection should receive at least one dose of MMR vaccine. This measure could help to close the immunisation gaps among adolescents and young adults in the EUR.

Acknowledgements

We would like to thank Nataša Berginc (Laboratory for Virology, National Institute of Public Health of Slovenia), Petra Kurzendörfer, Anne Wolbert, Christine Schwerdtfeger, Cornelia Lentz and Ingrid Deitemeier (Robert Koch Institute) for obtaining the laboratory results, and Drazen Stojanovic (Institute of Public Health of Ljubljana) and the staff of local public health offices in Sigmaringen, Zollernalbkreis, Freiburg, Tübingen, Mannheim, Heidelberg, Konstanz and Donauwörth for providing epidemiological information. We are also grateful to Kevin Brown and Richard Myers, Virus Reference Department, Public Health England, for providing the opportunity to use the Measles Nucleotide Surveillance database.

Conflict of interest

None declared.

Authors' contributions

All authors were involved in study design, collected samples and data, and contributed to data analysis and interpretation. Katarina Prosenc and Ondina Jordan Markocic conducted laboratory case confirmation and epidemiological investigation in Slovenia. Dorothee Lohr and Günter Pfaff were responsible for epidemiological investigation in the German Federal State of Baden-Wuerttemberg. Sabine Santibanez and Annette Mankertz conducted molecular-epidemiological investigation and analysis of antibody responses. All authors contributed to the manuscript and approved the final version.

References

1. World Health Organization (WHO). Surveillance Guidelines for Measles, Rubella and Congenital Rubella Syndrome in the WHO European Region. Copenhangen: WHO Regional Office for Europe;2012.
2. Mankertz A, Mulders MN, Shulga S, Kremer JR, Brown KE, Santibanez S, et al. Molecular genotyping and epidemiology of measles virus transmission in the World Health Organization European Region, 2007-2009. J Infect Dis. 2011;204 Suppl 1:S335-42. http://dx.doi.org/10.1093/infdis/jir101
3. Fiebelkorn AP, Goodson JL. Yellow book. Atlanta: Centers for Disease Control and Prevention; 2014. Chapter 3, Measles (Rubeola).
4. Botelho-Nevers E, Gautret P. Outbreaks associated to large open air festivals, including music festivals, 1980 to 2012. Euro Surveill. 2013;18(11):pii=20426.
5. Centers for Disease, Control Prevention (CDC). Multistate measles outbreak associated with an international youth sporting event--Pennsylvania, Michigan, and Texas, August-September 2007. MMWR Morb Mortal Wkly Rep. 2008;57(7):169-73.
6. Pfaff G, Lohr D, Santibanez S, Mankertz A, van Treeck U, Schonberger K, et al. Spotlight on measles 2010: Measles outbreak among travellers returning from a mass gathering, Germany, September to October 2010. Euro Surveill. 2010;15(50):pii=19750.
7. López Hernández B, Laguna Sorinas J, Marín Rodríguez I, Gallardo García V, Pérez Morilla E, Mayoral Cortés JM. Spotlight on measles 2010: An ongoing outbreak of measles in an unvaccinated population in Granada, Spain, October to November 2010: an ongoing outbreak of measles in an unvaccinated population in Granada, Spain, October to November 2010. Euro Surveill. 2010;15(50):pii=19746.
8. Expanded Programme on Immunization (EPI) team. Manual for the laboratory diagnosis of measles and rubella virus infection. 2nd ed. Geneva: World Health Organization (WHO); 2007.
9. Wichmann O, Siedler A, Sagebiel D, Hellenbrand W, Santibanez S, Mankertz A, et al. Further efforts needed to achieve measles elimination in Germany: results of an outbreak investigation. Bull World Health Organ. 2009;87(2):108-15. http://dx.doi.org/10.2471/BLT.07.050187
10. Grgic-Vitek M, Frelih T, Ucakar V, Prosenc K, Tomazic J, Petrovec M, et al. Spotlight on measles 2010: a cluster of measles in a hospital setting in Slovenia, March 2010. Euro Surveill. 2010;15(20):pii=19573.
11. Siedler A, Mankertz A, Feil F, Ahlemeyer G, Hornig A, Kirchner M, et al. Closer to the goal: efforts in measles elimination in Germany 2010. J Infect Dis. 2011;204 Suppl 1:S373-80. http://dx.doi.org/10.1093/infdis/jir068
12. Santibanez S, Tischer A, Heider A, Siedler A, Hengel H. Rapid replacement of endemic measles virus genotypes. J Gen Virol. 2002;83(Pt 11):2699-708.
13. Mankertz A, Mihneva Z, Gold H, Baumgarte S, Baillot A, Helble R, et al. Spread of measles virus D4-Hamburg, Europe, 2008-2011. Emerg Infect Dis. 2011;17(8):1396-401.
14. Measles virus nomenclature update: 2012. Wkly Epidemiol Rec. 2012;87(9):73-81.
15. Rota PA, Brown K, Mankertz A, Santibanez S, Shulga S, Muller CP, et al. Global distribution of measles genotypes and measles molecular epidemiology. J Infect Dis. 2011;204 Suppl 1:S514-23. http://dx.doi.org/10.1093/infdis/jir118
16. Fine PE. The interval between successive cases of an infectious disease. Am J Epidemiol. 2003;158(11):1039-47. http://dx.doi.org/10.1093/aje/kwg251
17. World Health Organization (WHO). Reported measles cases and incidence rates by WHO Member States 2011, 2012 as of 05 February 2013. Geneva: WHO; 2013.

18. Ministry of Health Italy. Piano Nazionale Prevenzione Vaccinale 2011-2013. Rome: Ministry of Health. Italian.
19. Robert Koch Institut (RKI). Impfquoten bei der Schuleingangsuntersuchung in Deutschland 2010. [Vaccination coverage at the primary school admission health checks in Germany 2010]. Epidemiologisches Bulletin. 2012;16. German.
20. European Centre for Disease Control and Prevention (ECDC). European monthly measles monitoring (EMMO). Issue 9: 19 Mar 2012. Stockholm: ECDC; 2012.
21. Richard JL, Masserey Spicher V. Large measles epidemic in Switzerland from 2006 to 2009: consequences for the elimination of measles in Europe. Euro Surveill. 2009;14(50):pii=19443.
22. Muscat M. Who gets measles in Europe? J Infect Dis. 2011;204 Suppl 1:S353-65. http://dx.doi.org/10.1093/infdis/jir067
23. Pannuti CS, Morello RJ, Moraes JC, Curti SP, Afonso AM, Camargo MC, et al. Identification of primary and secondary measles vaccine failures by measurement of immunoglobulin G avidity in measles cases during the 1997 Sao Paulo epidemic. Clin Diagn Lab Immunol. 2004;11(1):119-22.
24. Atrasheuskaya AV, Blatun EM, Neverov AA, Kameneva SN, Maksimov NL, Karpov IA, et al. Measles in Minsk, Belarus, 2001-2003: clinical, virological and serological parameters. J Clin Virol. 2005;34(3):179-85. http://dx.doi.org/10.1016/j.jcv.2004.11.024
25. Atrasheuskaya AV, Kulak MV, Neverov AA, Rubin S, Ignatyev GM. Measles cases in highly vaccinated population of Novosibirsk, Russia, 2000-2005. Vaccine. 2008;26(17):2111-8. http://dx.doi.org/10.1016/j.vaccine.2008.02.028
26. Kontio M, Jokinen S, Paunio M, Peltola H, Davidkin I. Waning Antibody Levels and Avidity: Implications for MMR Vaccine-Induced Protection. J Infect Dis. 2012;206(10):1542-8. http://dx.doi.org/10.1093/infdis/jis568
27. Hickman CJ, Hyde TB, Sowers SB, Mercader S, McGrew M, Williams NJ, et al. Laboratory characterization of measles virus infection in previously vaccinated and unvaccinated individuals. J Infect Dis. 2011;204 Suppl 1:S549-58. http://dx.doi.org/10.1093/infdis/jir106
28. Botelho-Nevers E, Cassir N, Minodier P, Laporte R, Gautret P, Badiaga S, et al. Measles among healthcare workers: a potential for nosocomial outbreaks. Euro Surveill. 2011;16(2):pii=19764.
29. Komitova R, Kunchev A, Mihneva Z, Marinova L. Nosocomial transmission of measles among healthcare workers, Bulgaria, 2010. Euro Surveill. 2011;16(15):pii=19842.
30. Vainio K, Steen TW, Arnesen TM, Rønning K, Ånestad G, Dudman S. Measles virus genotyping an important tool in measles outbreak investigation in Norway, 2011. Euro Surveill. 2012;17(50):pii=20340.
31. Gillesberg Lassen S, Schuster M, Stemmler M, Steinmüller A, Matysiak-Klose D, Mankertz A, et al. Measles outbreak spreading from the community to an anthroposophic school, Berlin, 2011. Epidemiol Infect. 2014;142(4):789-96. http://dx.doi.org/10.1017/S0950268813001398

Association between temperature, humidity and ebolavirus disease outbreaks in Africa, 1976 to 2014

S Ng (sophian@princeton.edu)[1], N E Basta[1], B J Cowling[2]
1. Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, United States
2. School of Public Health, The University of Hong Kong, Hong Kong, China

Citation style for this article:
Ng S, Basta NE, Cowling BJ. Association between temperature, humidity and ebolavirus disease outbreaks in Africa, 1976 to 2014. Euro Surveill. 2014;19(35):pii=20892. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20892

Article submitted on 04 September 2014 / published on 04 September 2014

Ebolavirus disease (EVD) outbreaks have been occurring sporadically in Central Africa since 1976. In 2014, the first outbreak in West Africa was reported in Guinea. Subsequent outbreaks then appeared in Liberia, Sierra Leone and Nigeria. The study of environmental factors underlying EVD epidemiology may provide useful insights into when and where EVD outbreaks are more likely to occur. In this paper, we aimed to investigate the association between climatic factors and onset of EVD outbreaks in humans. Our results suggest lower temperature and higher absolute humidity are associated with EVD outbreak onset in the previous EVD outbreaks in Africa during 1976 to 2014. Potential mechanisms through which climate may have an influence on ebolavirus infection in the natural host, intermediate hosts and humans are discussed. Current and future surveillance efforts should be supported to further understand ebolavirus transmission events between and within species.

Introduction

Ebolaviruses were first recognised as causing ebolavirus disease (EVD) in humans in outbreaks in South Sudan and the Democratic Republic of the Congo in Central Africa in 1976 [1,2]. The recent EVD outbreak in Guinea in 2014 is the first reported in West Africa [3]. Initial confirmed and probable cases in Liberia and Sierra Leone are reported to have travelled to Guinea [4]. These cases were followed by more extensive outbreaks in the two countries and later on a small number of ebolavirus disease cases were also detected in Nigeria. Genome sequencing analyses revealed that the Zaire ebolavirus causing the outbreak in Guinea was 97% identical to the Zaire ebolaviruses that had previously caused an outbreak in the Democratic Republic of the Congo and Gabon [5]. Phylogenetic analyses showed the virus isolated in Guinea belongs to a separate clade from previous Zaire ebolaviruses identified in the Democratic Republic of the Congo and Gabon [5]. Other ebolaviruses identified in previous EVD outbreaks in humans in Africa included the Sudan, Côte d'Ivoire and Bundibugyo species [1,6]. Despite the capacity of ebolaviruses to be transmitted between species, including humans, only sporadic outbreaks have been reported and most of them were limited to Central Africa. The spread of the current EVD outbreak outside central African countries to those in western Africa with a high volume of cross-border and international travel have raised concern regarding further spread to other countries within and outside Africa. Despite recent progress in human trials of treatment and vaccines, ebolavirus infections continue to pose a serious public health threat due to the high case fatality risk.

In some previous outbreaks, investigations revealed a clear connection between EVD and contact with the natural reservoir or infected intermediate hosts including bats, chimpanzees and other primates [1,7]. The European Centre for Disease Prevention and Control rapid risk assessment concluded direct contact with contaminated secretions, blood, organs and other bodily fluids of living or dead infected persons or animals or with objects heavily contaminated with such fluids have a high potential to lead to transmission [8]. EVD has also arisen as a result of importation of infected animals and laboratory contamination [1] but was not followed by sustained human-to-human transmission.

Seasonal and cyclical patterns of ebolavirus infections have been observed, suggesting seasonal changes in factors such as climate maybe useful predictors of EVD outbreaks [9,10]. Examination of these factors may also provide some insight into why EVD had been limited to central parts of Africa in the past and why it has started to appear in West Africa. The objective of this study was to investigate the association between climatic conditions and EVD outbreaks in Africa that occurred between 1976 and 2014, and to discuss potential mechanisms to which climate may have an influence on ebolavirus infection in the natural host, intermediate hosts and humans.

Table 1

Characteristics of human ebolavirus disease outbreaks in five African countries included in analyses of the effect of climatic conditions, 1976–2014

Country	Area	Onset of first outbreak	End of last outbreak	Years of climate data analysed	Ebolavirus species	Mean temperature in °C (SD)	Mean absolute humidity in kg/m³ (SD)
Guinea	Guékédou	Jan 2014	Ongoing	2013–2014[a]	Zaire	25.53 (0.96)	16.50 (1.55)
	Macenta	Jan 2014	Ongoing	2013–2014[a]	Zaire	24.79 (0.94)	15.22 (1.50)
	Kissidougou	Jan 2014	Ongoing	2013–2014[a]	Zaire	25.31 (1.08)	15.70 (2.04)
Gabon	Andock	Dec 1994	Feb 1995	1993–1995	Zaire	26.50 (1.08)	20.10 (1.27)
	Mayibout II	Jan 1996	Apr 1996	1995–1996	Zaire	24.80 (0.94)	18.38 (0.62)
	Booué	Jul 1996	Jan 1997	1995–1997	Zaire	26.37 (0.93)	19.76 (0.88)
	Ogooué-Ivindo	Oct 2001	Mar 2002	2000–2002	Zaire	25.38 (1.06)	18.86 (0.74)
Democratic Republic of the Congo	Bumba	Sep 1976	Oct 1976	1975–1976	Zaire	24.94 (0.63)	18.17 (0.35)
	Tandala	Jun 1977	Jun 1977	1976–1977	Zaire	25.50 (0.70)	18.67 (0.57)
	Kikwit	Jan 1995	Jul 1995	1994–1995	Zaire	25.18 (0.57)	17.55 (1.54)
	Cuvette-Ouest	Dec 2002	Dec 2003	2001–2003	Zaire	24.93 (0.89)	18.49 (0.74)
	Kasai Occidental	May 2007	Oct 2007	2006–2007	Zaire	24.84 (0.53)	18.21 (0.80)
	Mweka	Nov 2008	Jan 2009	2007–2009	Zaire	24.81 (0.55)	18.21 (0.80)
	Luebo	Nov 2008	Jan 2009	2007–2009	Zaire	24.44 (0.61)	17.75 (0.96)
	Isiro	Aug 2012	Oct 2012	2011–2012	Zaire	24.32 (1.36)	18.45 (0.97)
South Sudan	Nzara	Jun 1976	Oct 1979	1975–1979	Sudan	24.27 (0.98)	15.95 (1.57)
	Yambio	Apr 2004	Jun 2004	2003–2004	Sudan	26.05 (1.18)	17.51 (1.73)
Uganda	Gulu	Aug 2000	Jan 2001	1999–2001	Sudan	24.69 (1.26)	15.27 (1.59)
	Masindi	Aug 2000	Dec 2000	1999–2000	Sudan	23.17 (0.87)	15.15 (0.87)
	Mbarara	Aug 2000	Jan 2001	1999–2001	Sudan	19.79 (0.66)	13.00 (0.75)
	Bundibugyo	Aug 2007	Dec 2007	2006–2007	Bundibugyo	18.76 (1.16)	13.02 (0.81)
	Luwero	May 2011	Nov 2012	2010–2012	Sudan	23.27 (1.26)	15.54 (0.95)
	Kibaale	Jul 2012	Aug 2012	2011–2012	Sudan	25.19 (0.99)	16.20 (1.82)

SD: standard deviation.

[a] Climate data were available until 2012; climatic conditions in 2013 and 2014 were imputed from climate data during the previous three years.

Methods

Sources of data

A total of 28 reported EVD outbreaks in Africa were identified from records and references listed on the United States Centers for Disease Control and Prevention website [1]. These outbreaks have occurred in the Democratic Republic of the Congo, Congo, South Sudan, Gabon and Uganda since 1976, and recently in Guinea. Because this study is focused on emergence and local transmission of the viruses, two reports involving a medical professional who treated a case from an outbreak in Gabon and later travelled immediately to South Africa in 1996, and a scientist who performed an autopsy of a wild chimpanzee in Côte d'Ivoire in 1994 were excluded.

The onset of an outbreak is defined by the date of the first reported probable or laboratory-confirmed case. Climate data, including ambient temperature, vapour pressure and dew point, at the outbreak locations were obtained from the Climate Research Unit, University of East Anglia, United Kingdom [11]. Absolute humidity was calculated using the conversion formula published by the National Aeronautics and Space Administration [12].

Statistical methods

A distributed lag non-linear model (DLNM) developed by Gasparrini et al. [13] was used in our analyses to examine the association between climatic factors and EVD outbreaks [7]. DLNM was used since it allows for a non-linear exposure–response relationship and provides flexibility in modelling the time structure of the relationship. The time structure is specified so that the log odds of an EVD outbreak can vary between each lag period following exposure of humans and intermediate host and natural host populations to certain climatic conditions at each outbreak area.

An earlier report of a detailed EVD outbreak investigation suggested exposure of the first cases to infected bats might precede detected outbreaks in humans by three months [7]. Lagged effects of one month, two months and three months were considered when our

Figure 1

Geographical distribution of human ebolavirus disease outbreaks included in analyses of monthly temperature and absolute humidity, 1976–2014

The red circles represent the outbreak areas.

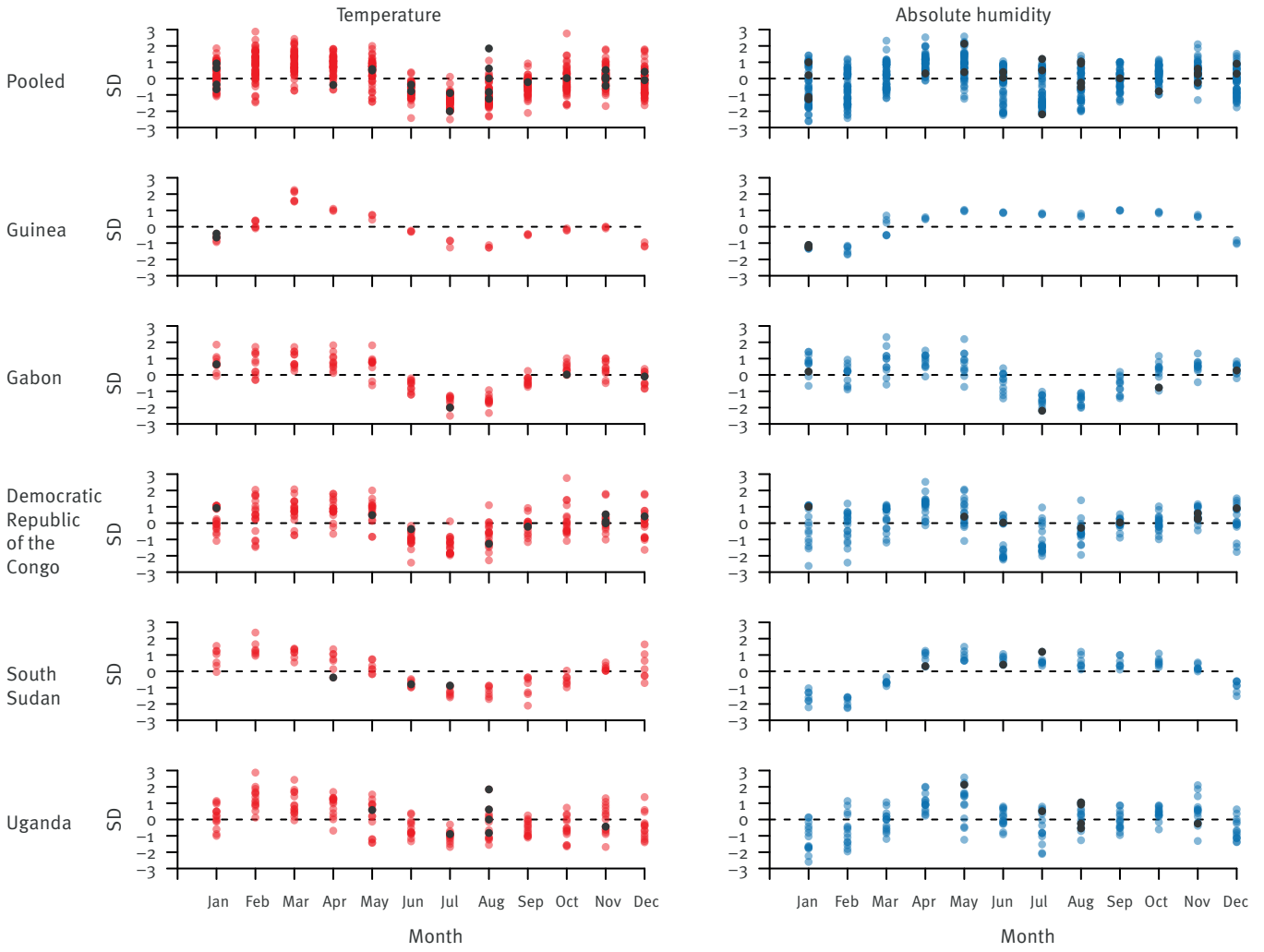
models were fitted. The relationship between exposure variables (monthly temperature and absolute humidity) and the log odds of response (onset of an EVD outbreak in humans) were specified using first (linear) and second (quadratic) degree orthogonal polynomials in binomial regression models. Likewise, zeroth (uniform), first (linear) and second (quadratic) degree orthogonal polynomials were used to specify the time structure of the exposure–response relationship.

Climate data were available until 2012 and the monthly temperature and absolute humidity in 2013 and 2014 were imputed using the mean same month observations during the previous three years. Climate data for Gabon was used for one outbreak that occurred at the border between Congo and Gabon.

Annual climate data from the year before the first outbreak to the year of the last outbreak at each outbreak area were analysed. Odds ratios of EVD outbreaks associated with deviation from mean climatic conditions over these years were calculated. The standardised monthly temperature and absolute humidity were visualised and variance inflation factors were calculated to inspect for multicollinearity between the two explanatory variables. The standardisation was carried out within each of the smallest geographical jurisdictions described in the outbreak reports. Separate models

Figure 2

Standardised monthly temperature and absolute humidity in five African countries with human ebolavirus disease outbreaks[a], 1976–2014



SD: standard deviation.

Standardised monthly temperature (red circles) and absolute humidity (blue circles) are shown.

The observations during onset months of ebolavirus disease outbreaks are highlighted as black circles. The mean and standard deviation of temperature and absolute humidity and the years of climate data included in the analyses for each outbreak area can be found in Table 1.

[a] Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda. An outbreak occurring at the border of the Republic of Congo and Gabon was included here as an outbreak in Gabon.

were used for temperature and absolute humidity when evidence of multicollinearity was observed.

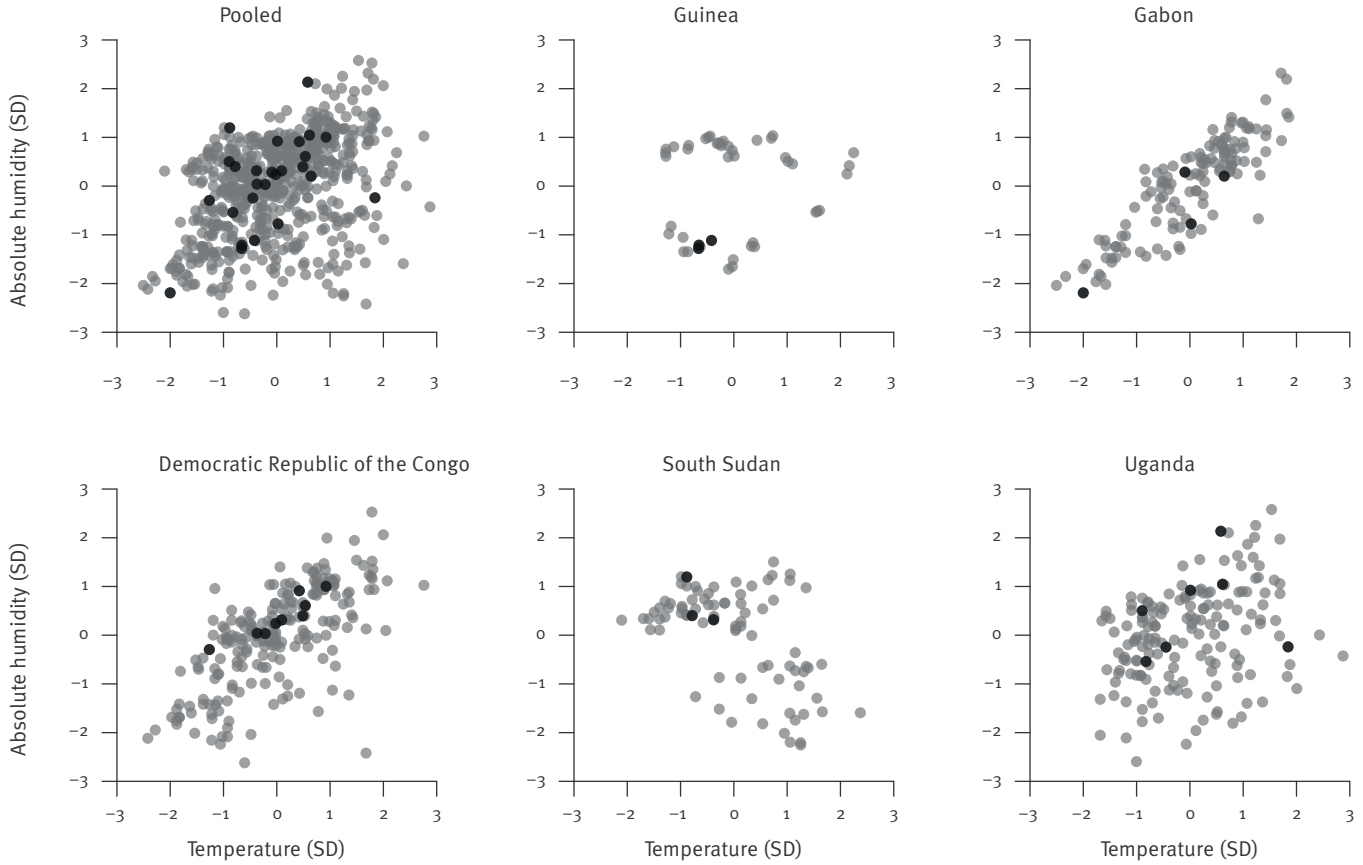
General estimating equations (GEE) [14] were used to adjust for correlations between multiple observations within the smallest geographical jurisdiction described in the outbreak reports. Both pooled and stratified analyses were performed for the five outbreak countries: the Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda. Quasilikelihood under the independence model criterion (QIC) is a modification of Akaike's information criterion (AIC) for models using GEE [15]. All model specifications were evaluated using QIC and the final models with the lowest QIC were selected.

Results

The geographical distribution of EVD outbreaks in the Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda is shown in Figure 1. The mean and standard deviation of monthly temperature and absolute humidity, causative ebolavirus species and outbreak period in each outbreak area are listed in Table 1 [1,6,16]. In Guinea, the mean temperature was rather similar to that of other areas with outbreaks of Zaire ebolavirus disease (in the Democratic Republic of the Congo and Gabon), while the mean absolute

Figure 3

Correlation between standardised mean monthly temperature and absolute humidity in five African countries with human ebolavirus disease outbreaks[a], 1976–2014



SD: standard deviation.

The correlation between standardised mean monthly temperature and absolute humidity is shown as grey circles. The observations during onset months of ebolavirus disease outbreaks are highlighted as black circles. The years of climate data included in the analyses for each outbreak area can be found in Table 1.

[a] Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda. An outbreak occurring at the border of the Republic of Congo and Gabon was included here as an outbreak in Gabon.

humidity was lower, as in areas where previous outbreaks of Sudan ebolavirus disease occurred (Sudan and Uganda) [1]. Standardised monthly temperature and absolute humidity in the five countries analysed are shown in Figure 2. Consistent patterns in annual variation of temperature were observed across the five countries: June to August was generally cooler than the mean with February to April being the warmer months. The annual pattern of absolute humidity was, however, less consistent between countries. While the absolute humidity remained above the mean from April to November in Guinea and South Sudan, only March and April were more humid in the other three countries. In Gabon, July and August were noticeably drier, but this was not seen elsewhere. Since temperature and absolute humidity in the Democratic Republic of the Congo and Gabon were highly positively correlated (Figure 3), separate models were used in analysing their correlation with EVD outbreak onset. The variance inflation factors were low in Guinea, South Sudan, Uganda and in the pooled analyses, therefore temperature and absolute humidity were included as covariates in the same models.

In the pooled analysis, the best-fitting model specified a uniform exposure–response relationship across the two months' lag period for temperature and the three months' lag period for absolute humidity (Table 2). Lower temperature and higher humidity (standard deviation) were found to log-linearly associate with increased risk of human EVD outbreak onset during each month in the lag periods. The estimated cumulative log odds ratio of human EVD outbreak onset at each month following exposure of humans and intermediate host and natural host populations to certain climatic conditions are shown in Figure 4. These associations were shown to be statistically significant across the entire lag period (Tables 3 and 4). Analyses stratified by country were underpowered and analyses only including areas with Zaire ebolavirus outbreaks

Table 2

Model specifications of the best-fitting models selected based on quasilikelihood under the independence model criterion, in five African countries with human ebolavirus outbreaks, 1976–2014

Country and outbreaks by ebolavirus species	Lag period following exposure[a] to temperature (in months)	Lag period following exposure[a] to absolute humidity (in months)	Degree of orthogonal polynomial used to specify the relationship between temperature and the log odds of EVD outbreak	Degree of orthogonal polynomial used to specify the relationship between absolute humidity and the log odds of EVD outbreak	How odds ratio vary across lag period following exposurea to temperature	How odds ratio vary across lag period following exposurea to absolute humidity
Country						
Pooled	2	3	1st	1st	Uniform	Uniform
Guinea[b]	–	–	–	-	–	–
Gabon[c]	2	NA	1st	NA	Uniform	NA
Gabon[d]	NA	2	NA	1st	NA	Uniform
Democratic Republic of the Congo[c]	2	NA	1st	NA	Uniform	NA
Democratic Republic of the Congo[d]	NA	1	NA	2nd	NA	Uniform
South Sudan	1	1	1st	1st	Uniform	Uniform
Uganda	2	3	1st	2nd	Uniform	Uniform
Ebolavirus species						
Zaire species outbreaks	2	3	1st	1st	Uniform	Uniform
Sudan species outbreaks	2	3	1st	1st	Uniform	Uniform
Bundibugyo species outbreaks[b]	–	–	–	–	–	–

EVD: ebolavirus disease; NA: not applicable.

[a] Exposure of humans and intermediate host and natural host populations.

[b] The analysis was not performed due to insufficient number of outbreaks.

[c] Model including temperature as explanatory variable.

[d] Model including absolute humidity as explanatory variable.

produced consistent conclusions (Figure 5). Stratified analyses for Sudan and Bundibugyo species were underpowered. The specifications of the best-fitting stratified models can be found in Table 2.

Discussion

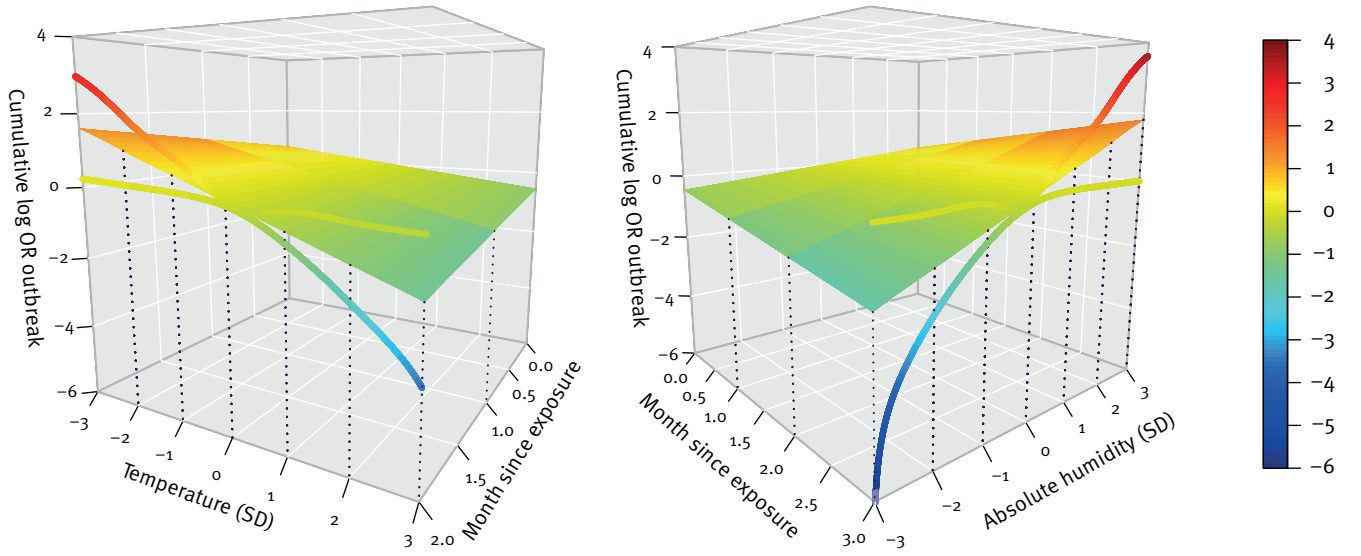
Our analyses of human EVD outbreaks in Africa suggest that the onset of these outbreaks was associated with conditions with higher absolute humidity and lower temperature when their time-lagged effects are taken into account. This is one of the first studies to examine the association between climatic factors and EVD outbreaks in humans. Our findings are consistent with the prediction of previous ecological niche models that ebolaviruses are more likely to be distributed in areas of humid Afrotropic rainforests where the temperature is moderate [17]. Previous EVD outbreaks in humans have been observed in both dry and wet seasons [9,10,18-20]. This is consistent with our analysis (Figure 2), which shows that when the time-lagged effect of environmental exposure is not considered, EVD outbreaks do not have a clear association with temperature and humidity.

On the basis of knowledge of ebola-related viruses, there has been speculation that plants, arthopods, bats and many other animals could be the natural host for ebolaviruses [21]. However, to date, evidence of potential ebolavirus persistence has only been found in bats [22]. Further animal virological studies are required to identify and verify all natural host species for ebolaviruses. Although seasonal patterns of ebolavirus infections among bats and other potential natural hosts have not been fully characterised, seroprevalence studies in bats have found the highest rates of seropositivity among adults and pregnant females [23]. This finding leads to the postulation that fighting and mating among bats may be associated with ebolavirus transmission [23]. These behaviours have been documented to be most frequent during rainy or wet seasons [24]: this may partly explain how climatic factors are associated with ebolavirus infection risk among bats, one of the potential natural hosts.

Viral persistence studies in EVD patients have found the virus to be more persistent in semen than in other bodily fluids and fomites [25]. Ebolavirus was found to remain detectable in semen for up to 91 days [26]. This finding highlights the relative importance of sexual transmission, if virus shedding in bats follows a similar pattern.

Figure 4

Estimated cumulative odds ratios of onset of human ebolavirus disease outbreaks at each month following exposure of humans and intermediate host and natural host populations to certain climatic conditions in five African countries with human ebolavirus disease outbreaks[a], 1976–2014



CI: confidence interval; OR: cumulative odds ratio; SD: standard deviation.

The lag period was two months for the effect of temperature and three months for absolute humidity. The OR was calculated with reference to ebolavirus disease outbreak onset risk at mean temperature/absolute humidity conditions. The 95% CIs for the estimated cumulative log OR at the end of the lag period are shown as the two lines enclosing the surface that shows the cumulative log OR. The mean and standard deviation of temperature and absolute humidity, and the years of climate data included in the analyses for each outbreak area can be found in Table 1. Numerical values for all ORs and 95% CIs can be found in Tables 3 and 4.

[a] Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda. An outbreak occurring at the border of the Republic of Congo and Gabon was included here as an outbreak in Gabon.

Table 3

Estimated cumulative odds ratio of onset of human ebolavirus disease outbreaks at each month following exposure to temperature conditions[a], 1976–2014

Temperature (SD)	Same month	First month	Second month
	OR (95% CI)	OR (95% CI)	OR (95% CI)
−3	1.71 (1.08–2.70)	2.93 (1.17–7.29)	5.00 (1.27–19.68)
−2	1.43 (1.06–1.94)	2.05 (1.11–3.76)	2.93 (1.17–7.29)
−1	1.20 (1.03–1.39)	1.43 (1.06–1.94)	1.71 (1.08–2.70)
0	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
1	0.84 (0.72–0.97)	0.70 (0.52–0.95)	0.58 (0.37–0.92)
2	0.70 (0.52–0.95)	0.49 (0.27–0.90)	0.34 (0.14–0.85)
3	0.58 (0.37–0.92)	0.34 (0.14–0.85)	0.20 (0.05–0.79)

CI: confidence interval; OR: cumulative odds ratio; SD: standard deviation.

[a] Estimated from the best-fitting model for the pooled analyses of outbreaks caused by Zaire, Sudan and Bundibugyo ebolaviruses in Guinea, Gabon, Democratic Republic of the Congo, South Sudan and Uganda. The outbreak areas and time period included in the analyses are described in Table 1. The best-fitting model included two months as the duration of the lag effect.

Seasonal migration of fruit bats may result in increased contact with humans and other animals [10]. An outbreak investigation in the Democratic Republic of the Congo in 2007 linked the first human case to migratory bats that stayed in the area during the migratory season [7]. Further investigation should be carried out to study whether disruption/change in migratory route or virus acquisition in other bat species with a different geographical range would explain the first outbreak in West Africa. Bats host many viruses that are highly pathogenic in other mammals [27]. It has been hypothesised that flight activities maintain a high body temperature and metabolic rate, which may mimic the effect of a febrile immune response in limiting virulence of a virus that may otherwise be highly pathogenic [27,28]. Seasonal environmental and behavioural

Table 4

Estimated cumulative odds ratios of onset of human ebolavirus disease outbreaks at each month following exposure to absolute humidity conditions[a], 1976–2014

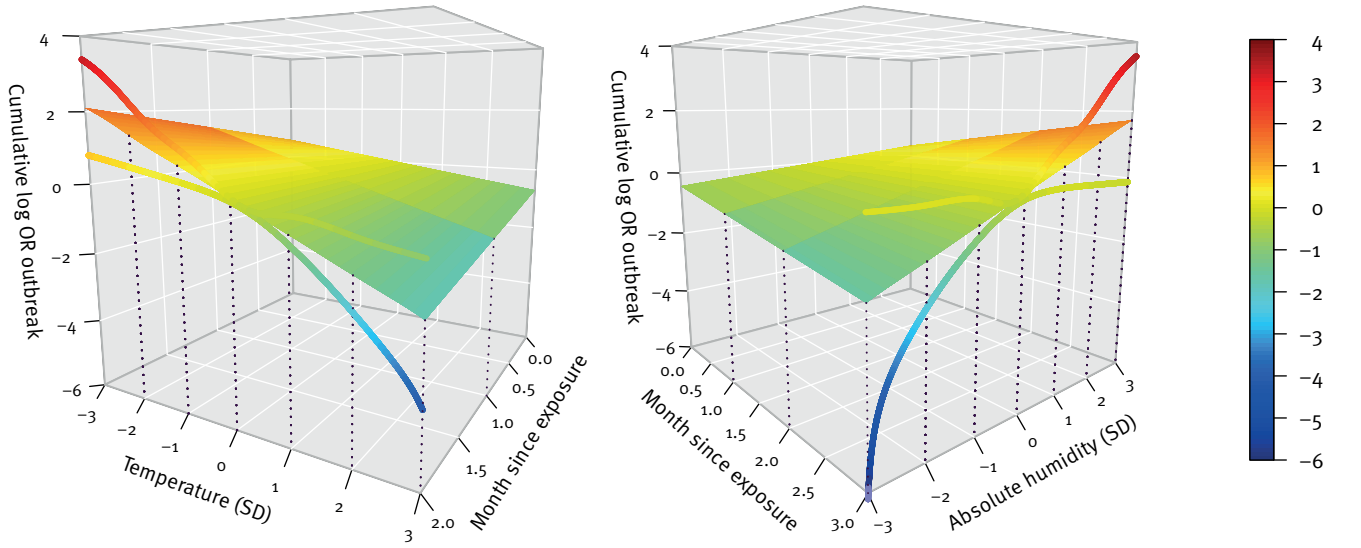
Absolute humidity (SD)	Same month	First month	Second month	Third month
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
−3	0.64 (0.40–1.00)	0.40 (0.16–1.00)	0.26 (0.07–1.00)	0.16 (0.03–1.00)
−2	0.74 (0.55–1.00)	0.55 (0.30–1.00)	0.40 (0.16–1.00)	0.30 (0.09–1.00)
−1	0.86 (0.74–1.00)	0.74 (0.55–1.00)	0.64 (0.40–1.00)	0.55 (0.30–1.00)
0	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	1.00 (1.00–1.00)
1	1.16 (1.00–1.35)	1.35 (1.00–1.83)	1.57 (1.00–2.47)	1.83 (1.00–3.34)
2	1.35 (1.00–1.83)	1.83 (1.00–3.34)	2.47 (1.00–6.11)	3.34 (1.00–11.18)
3	1.57 (1.00–2.47)	2.47 (1.00–6.11)	3.88 (1.00–15.11)	6.10 (1.00–37.37)

CI: confidence interval; OR: cumulative odds ratio; SD: standard deviation.

[a] Estimated from the best-fitting model for the pooled analyses of outbreaks caused by Zaire, Sudan and Bundibugyo ebolaviruses in Guinea, Gabon, Democratic Republic of the Congo, South Sudan and Uganda. The outbreak areas and time period included in the analyses are described in Table 1. The best-fitting model included three months as the duration of the lag effect.

Figure 5

Estimated cumulative odds ratios of onset of human Zaire ebolavirus disease outbreaks at each month following exposure of humans and intermediate host and natural host populations to certain climatic conditions in three African countries with human Zaire ebolavirus disease outbreaks[a], 1976–2014



CI: confidence interval; OR: cumulative odds ratio; SD: standard deviation.

The lag period was two months for the effect of temperature and three months for absolute humidity. The OR was calculated with reference to Zaire ebolavirus disease outbreak onset risk at mean temperature/absolute humidity conditions. The 95% CIs for the estimated cumulative log OR at the end of the lag period are shown as the two lines enclosing the surface that shows the cumulative log OR. The mean and standard deviation of temperature and absolute humidity, and the years of climate data included in the analyses for each outbreak area can be found in Table 1. Numerical values for all ORs and 95% CIs can be found in Tables 3 and 4.

[a] Democratic Republic of the Congo, Gabon, Guinea, South Sudan and Uganda. An outbreak occurring at the border of the Republic of Congo and Gabon was included here as an outbreak in Gabon.

factors such as long migratory flight may influence body temperature and metabolic rate in bats. This may result in altered susceptibility to and severity of ebolavirus infection. Reduction in susceptibility and severity may have bidirectional effects on ebolavirus transmission dynamics. While less severe infections may allow infected bats to remain active in transmitting the virus, reduction in susceptibility may reduce the overall infection rate among the bat population.

Peaks in mortality due to EVD in chimpanzees, gorillas and duikers (a type of antelope) were observed to coincide with some of the previous human EVD outbreaks [29]. EVD outbreaks in non-human primates have mostly been reported to occur at the end of rainy seasons [10,30,31]: however, it has been unclear whether this was due to earlier humid conditions or current dry conditions. As in bats, the behaviour of non-human primates and their exposure to bats may vary with the

season. A study of chimpanzees in Côte d'Ivoire found that they made a higher number of kills per day when hunting in the wet seasons [32]. This may lead to a sudden increase in consumption or contact with prey that is a natural reservoir of ebolaviruses. Furthermore, increased social mixing during wet seasons may also facilitate transmission of ebolaviruses among chimpanzees [24]. Similarly, human-to-human and human-to-animal contact patterns may have a seasonal effect on the risk of an EVD outbreak in humans.

While natural hosts such as bats can serve as a common source of cross-species transmission for humans and other primates, non-human primates can act as intermediate hosts in zoonotic events that result in human infections. Therefore, the time frame of the spillover effect of environmental exposure in the natural hosts may depend on the transmission chain of the zoonotic events and how these events are associated with climate. Environmental exposure may also have transient, immediate effects on the susceptibility to and severity of ebolavirus infection among natural hosts, intermediate hosts and humans. Previous experimental studies have found human exposure to low temperature may trigger changes in the immune response [33-36]. If these effects are conserved between these hosts, periods of suitable climatic conditions may provide windows of opportunity for cross-species transmission to occur. Serosurveillance studies in human populations in Africa have revealed a much higher prevalence of ebolavirus antibodies than the attack rate reported in previous EVD outbreaks [37,38]. This may suggest that exposure of humans to ebolaviruses or other cross-reactive pathogens was more prevalent than previously thought. In fact, a study of contacts of EVD patients has revealed that some ebolavirus infections can be mild or asymptomatic [39]. Tissue tropism of ebolaviruses has been studied: the viruses were found to target and infect immune cells including monocytes, macrophages and immature dendritic cells and to cause highly pathological immune responses [40,41]. Further studies should characterise how previously identified environmental effects on the immune response [33-36] may translate to ebolavirus infection outcomes. Since EVD cases with milder symptoms are more likely to be under-reported, the observed EVD outbreak pattern may in part be attributable to seasonal differences in EVD severity.

In the past, EVD outbreaks were confined to the central African countries and it is essential to understand why EVD has appeared in West Africa. This will have implications on how likely it is that EVD outbreaks will occur in the rest of the world. Climate has been found in our study to be associated with EVD outbreaks and, as discussed in this paper, there are a number of ways in which climate could be associated with the seasonal risk factors of EVD outbreaks. Further studies should investigate the potential impact of climate change on the geographical boundary of the virus and the time period in which EVD is likely to occur.

There are a number of limitations in this study. The initial identification of EVD outbreaks in Africa has mostly been reliant on the clinical manifestation of cases; however, some EVD cases presented with non-specific symptoms that can be easily confused with other diseases that are endemic in Africa [42]. Due to the limited resources and remoteness of some of the rural areas where human–animal contacts are most frequent, some EVD outbreaks might be under-detected. Delayed detection of EVD outbreaks should be expected and we addressed this by using distributed time-lag models. However, it is still difficult to interpret or construct the time structure of the exposure–response relationship since little information on reporting delays is available. The incubation period of EVD may be up to 21 days or more [43], and this has to be taken into account when interpreting time-lagged effects of environmental exposures. The choice of time structure of the exposure–response relationship is based on the model best fitting our data. Given that EVD outbreaks in humans are rather rare, our study may be underpowered to investigate a more sophisticated time structure of the exposure–response relationship and to detect a non-linear exposure–response relationship. The earliest few human cases are likely to be under-detected and our study may have excluded smaller EVD outbreaks that were unreported. While climatic variation can be a useful predictor, its association with EVD outbreaks may depend on other ecological and environmental factors, as well as on natural host species that vary between geographical areas. Our findings may therefore only apply to areas that share similar characteristics with the outbreak areas included in the analyses. It is most likely that ecological and environmental differences also exist between outbreak areas included the analyses. Our country-specific analyses were unfortunately underpowered, as EVD outbreaks are rare. It is also possible that the association between climate and EVD is specific to ebolavirus species. Our study was only able to provide estimates for Zaire ebolavirus outbreaks since there were fewer outbreaks due to other ebolavirus species.

In order to understand the transmission dynamics of ebolavirus, current efforts in identifying the natural and intermediate hosts of ebolaviruses should be continued and supported. A better understanding of the chain of transmission from the natural reservoir to humans is essential for characterising the epidemiology of ebolavirus infections and directing public health preventive policies. Longitudinal serological and virological surveillance studies will help in identifying the event sequence and interfaces that are important for outbreaks in humans. Our study focused on the onset of EVD outbreaks, as we aimed to investigate environmental factors that are associated with cross-species transmission. To enable factors associated with human-to-human transmission to be investigated, current support to the outbreak countries in case detection and reporting should be continued.

Conflict of interest

BJC reports receiving research funding from MedImmune Inc and consults for Crucell NV. The authors report no other potential conflicts of interest.

Authors' contributions

SN, NEB and BJC have each participated in the conception, design, analysis and writing of the manuscript.

References

1. Centers for Disease Control and Prevention (CDC). Outbreaks chronology: Ebola hemorrhagic fever. Atlanta, GA: CDC. Updated 26 Mar 2014. [Accessed 28 Mar 2014]. Available from: http://www.cdc.gov/vhf/ebola/resources/outbreak-table.html
2. Peters CJ, LeDuc JW. An introduction to Ebola: the virus and the disease. J Infect Dis. 1999;179 Suppl 1:ix-xvi. http://dx.doi.org/10.1086/514322
3. World Health Organization (WHO) Regional Office for Africa. Ebola virus disease, Liberia (Situation as of 30 March 2014). Brazzaville: WHO Regional Office for Africa. Updated 30 Mar 2014. [Accessed 31 Mar 2014]. Available from: http://www.afro.who.int/en/clusters-a-programmes/dpc/epidemic-a-pandemic-alert-and-response/outbreak-news/4072-ebola-haemorrhagic-fever-liberia.html
4. Bagcchi S. Ebola haemorrhagic fever in west Africa. Lancet Infect Dis. 2014;14(5):375. http://dx.doi.org/10.1016/S1473-3099(14)70034-9
5. Baize S, Pannetier D, Oestereich L, Rieger T, Koivogui L, Magassouba N, et al. Emergence of Zaire Ebola virus disease in Guinea - preliminary report. N Engl J Med. 2014 Apr 16. [Epub ahead of print]. http://dx.doi.org/10.1056/NEJMoa1404505
6. Muyembe-Tamfum JJ, Mulangu S, Masumu J, Kayembe JM, Kemp A, Paweska JT. Ebola virus outbreaks in Africa: past and present. Onderstepoort J Vet Res. 2012;79(2):451. http://dx.doi.org/10.4102/ojvr.v79i2.451
7. Leroy EM, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, et al. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. Vector Borne Zoonotic Dis. 2009;9(6):723-8. http://dx.doi.org/10.1089/vbz.2008.0167
8. European Centre For Disease Prevention and Control (ECDC). Rapid risk assessment. Outbreak of ebola virus disease in West Africa. Third update, 1 August 2014. ECDC: Stockholm; 2014. Available from: http://ecdc.europa.eu/en/publications/Publications/ebola-outbreak-west-africa-1-august-2014.pdf
9. Pinzon JE, Wilson JM, Tucker CJ, Arthur R, Jahrling PB, Formenty P. Trigger events: enviroclimatic coupling of Ebola hemorrhagic fever outbreaks. Am J Trop Med Hyg. 2004;71(5):664-74.
10. Bausch DG, Schwarz L. Outbreak of ebola virus disease in Guinea: where ecology meets economy. PLoS Negl Trop Dis. 2014;8(7):e3056. http://dx.doi.org/10.1371/journal.pntd.0003056
11. Harris I, Jones PD, Osborn TJ, Lister DH. Updated high-resolution grids of monthly climatic observations – the CRU TS3.10 Dataset. Int J Climatol. 2013;34(3):623-42. Epub 21 May 2013. http://dx.doi.org/10.1002/joc.3711
12. Parish OO, Putnam TW. Equations for the determination of humidity from dewpoint and psychrometric data. Washington, DC: National Aeronautics and Space Administration (NASA); 1977. NASA Technical Note D-8401. Available from: http://www.nasa.gov/centers/dryden/pdf/87878main_H-937.pdf
13. Gasparrini A, Armstrong B, Kenward MG. Distributed lag non-linear models. Stat Med. 2010;29(21):2224-34. http://dx.doi.org/10.1002/sim.3940
14. Liang K-Y, Zeger SL. Longitudinal data analysis using generalized linear models. Biometrika. 1986;73(1):13-22. http://dx.doi.org/10.1093/biomet/73.1.13
15. Hardin JW, Hilbe JM. Generalized estimating equations. 2nd ed. Boca Raton, FL: Chapman and Hall/CRC Press; 2012 Available from: http://www.crcnetbase.com/isbn/978-1-4398-8113-2
16. Wittmann TJ, Biek R, Hassanin A, Rouquet P, Reed P, Yaba P, et al. Isolates of Zaire ebolavirus from wild apes reveal genetic lineage and recombinants. Proc Natl Acad Sci U S A. 2007;104(43):17123-7. http://dx.doi.org/10.1073/pnas.0704076104
17. Peterson AT, Bauer JT, Mills JN. Ecologic and geographic distribution of filovirus disease. Emerg Infect Dis. 2004;10(1):40-7. http://dx.doi.org/10.3201/eid1001.030125
18. Johnson BK, Wambui C, Ocheng D, Gichogo A, Oogo S, Libondo D, et al. Seasonal variation in antibodies against Ebola virus in Kenyan fever patients. Lancet. 1986;1(8490):1160. http://dx.doi.org/10.1016/S0140-6736(86)91876-3
19. Busico KM, Marshall KL, Ksiazek TG, Roels TH, Fleerackers Y, Feldmann H, et al. Prevalence of IgG antibodies to Ebola virus in individuals during an Ebola outbreak, Democratic Republic of the Congo, 1995. J Infect Dis. 1999;179 Suppl 1:S102-7. http://dx.doi.org/10.1086/514309
20. Groseth A, Feldmann H, Strong JE. The ecology of Ebola virus. Trends Microbiol. 2007;15(9):408-16. http://dx.doi.org/10.1016/j.tim.2007.08.001
21. Monath TP. Ecology of Marburg and Ebola viruses: speculations and directions for future research. Journal Infect Dis. 1999;179 Suppl 1:S127-38. http://dx.doi.org/10.1086/514281
22. Olival KJ, Islam A, Yu M, Anthony SJ, Epstein JH, Khan SA, et al. Ebola virus antibodies in fruit bats, Bangladesh. Emerg Infect Dis. 2013;19(2):270-3. http://dx.doi.org/10.3201/eid1902.120524
23. Pourrut X, Delicat A, Rollin PE, Ksiazek TG, Gonzalez JP, Leroy EM. Spatial and temporal patterns of Zaire ebolavirus antibody prevalence in the possible reservoir bat species. J Infect Dis. 2007;196 Suppl 2:S176-83. http://dx.doi.org/10.1086/520541
24. Doran D. Influence of seasonality on activity patterns, feeding behavior, ranging, and grouping patterns in Taï chimpanzees. Int J Primatol. 1997;18(2):183-206. http://dx.doi.org/10.1023/A:1026368518431
25. Bausch DG, Towner JS, Dowell SF, Kaducu F, Lukwiya M, Sanchez A, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. J Infect Dis. 2007;196 Suppl 2:S142-7. http://dx.doi.org/10.1086/520545
26. Rowe AK, Bertolli J, Khan AS, Mukunu R, Muyembe-Tamfum JJ, Bressler D, et al. Clinical, virologic, and immunologic follow-up of convalescent Ebola hemorrhagic fever patients and their household contacts, Kikwit, Democratic Republic of the Congo. Commission de Lutte contre les Epidemies a Kikwit. J Infect Dis. 1999;179 Suppl 1:S28-35. http://dx.doi.org/10.1086/514318
27. O'Shea TJ, Cryan PM, Cunningham AA, Fooks AR, Hayman DT, Luis AD, et al. Bat flight and zoonotic viruses. Emerg Infect Dis. 2014;20(5):741-5. http://dx.doi.org/10.3201/eid2005.130539
28. Zhang G, Cowled C, Shi Z, Huang Z, Bishop-Lilly KA, Fang X, et al. Comparative analysis of bat genomes provides insight into the evolution of flight and immunity. Science. 2013;339(6118):456-60. http://dx.doi.org/10.1126/science.1230835
29. Rouquet P, Froment JM, Bermejo M, Kilbourn A, Karesh W, Reed P, et al. Wild animal mortality monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001-2003. Emerg Infect Dis. 2005;11(2):283-90. http://dx.doi.org/10.3201/eid1102.040533
30. Lahm SA, Kombila M, Swanepoel R, Barnes RF. Morbidity and mortality of wild animals in relation to outbreaks of Ebola haemorrhagic fever in Gabon, 1994-2003. Trans R Soc Trop Med Hyg. 2007;101(1):64-78. http://dx.doi.org/10.1016/j.trstmh.2006.07.002
31. Bermejo M, Rodriguez-Teijeiro JD, Illera G, Barroso A, Vilà C, Walsh PD. Ebola outbreak killed 5000 gorillas. Science. 2006;314(5805):1564. http://dx.doi.org/10.1126/science.1133105
32. Kuhn JH. Ecology of filoviruses: search for reservoirs. In: Calisher CH, editor. Filoviruses: a compendium of 40 years of epidemiological, clinical, and laboratory studies. New York, NY: Springer Vienna; 2008. p. 153-70.
33. Fairchild KD, Viscardi RM, Hester L, Singh IS, Hasday JD. Effects of hypothermia and hyperthermia on cytokine production by cultured human mononuclear phagocytes from adults and newborns. JInterferon Cytokine Res. 2000;20(12):1049-55. http://dx.doi.org/10.1089/107999000750053708
34. Mace TA, Zhong L, Kilpatrick C, Zynda E, Lee CT, Capitano M, et al. Differentiation of CD8+ T cells into effector cells is enhanced by physiological range hyperthermia. J Leukoc Biol. 2011;90(5):951-62. http://dx.doi.org/10.1189/jlb.0511229
35. Brenner IK, Castellani JW, Gabaree C, Young AJ, Zamecnik J, Shephard RJ, et al. Immune changes in humans during cold exposure: effects of prior heating and exercise. J Appl Physiol. 1999;87(2):699-710.
36. Nelson RJ, Demas GE, Klein SL, Kriegsfeld LK, Bronson F. Seasonal patterns of stress, immune function, and disease. New York, NY: Cambridge University Press; 2002. http://dx.doi.org/10.1017/CBO9780511546341
37. Gonzalez JP, Josse R, Johnson ED, Merlin M, Georges AJ, Abandja J, et al. Antibody prevalence against haemorrhagic fever viruses in randomized representative Central African populations. Res Virol. 1989;140(4):319-31. http://dx.doi.org/10.1016/S0923-2516(89)80112-8

38. Becquart P, Wauquier N, Mahlakoiv T, Nkoghe D, Padilla C, Souris M, et al. High prevalence of both humoral and cellular immunity to Zaire ebolavirus among rural populations in Gabon. PloS One. 2010;5(2):e9126. http://dx.doi.org/10.1371/journal.pone.0009126

39. Leroy EM, Baize S, Volchkov VE, Fisher-Hoch SP, Georges-Courbot MC, Lansoud-Soukate J, et al. Human asymptomatic Ebola infection and strong inflammatory response. Lancet. 2000;355(9222):2210-5. http://dx.doi.org/10.1016/S0140-6736(00)02405-3

40. Zampieri CA, Sullivan NJ, Nabel GJ. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nat Immunol. 2007;8(11):1159-64. http://dx.doi.org/10.1038/ni1519

41. Wong G, Kobinger GP, Qiu X. Characterization of host immune responses in Ebola virus infections. Expert Rev Clin Immunol. 2014;10(6):781-90. http://dx.doi.org/10.1586/1744666X.2014.908705

42. Centers for Disease Control and Prevention (CDC). Signs and symptoms. Ebola hemorrhagic fever. Atlanta, GA: CDC. [Accessed 3 Aug 2014]. Available from: http://www.cdc.gov/vhf/ebola/symptoms/

43. Eichner M, Dowell SF, Firese N. Incubation period of ebola hemorrhagic virus subtype Zaire. Osong Public Health Res Perspect. 2011;2(1):3-7. http://dx.doi.org/10.1016/j.phrp.2011.04.001

Is it reasonable to abandon obligatory vaccinations in Italy? A 2013 survey

C P Pelullo[1], S Marino[1], A J Valdes Abuadili[1], G Signoriello[2], F Attena (francesco.attena@unina2.it)[3]
1. School of Hygiene and Preventive Medicine of the Second University of Naples, Naples, Italy
2. Medical Statistics Unit of the Second University of Naples, Naples, Italy
3. Department of Experimental Medicine of the Second University of Naples, Naples, Italy

Citation style for this article:
Pelullo CP, Marino S, Valdes Abuadili AJ, Signoriello G, Attena F. Is it reasonable to abandon obligatory vaccinations in Italy? A 2013 survey. Euro Surveill. 2014;19(35):pii=20889. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=20889

Article submitted on 30 January 2014 / published on 04 September 2014

In Italy, infant vaccinations are mandatory for four infectious diseases: diphtheria, polio, tetanus and hepatitis B. In the past, there was widespread apprehension in Italy that doing away with obligatory vaccinations would reduce the coverage rate, but the possibility of making vaccinations optional has recently become more popular. The objectives of this study were to investigate parental willingness to vaccinate their children if those vaccinations were no longer mandatory and to evaluate the variables influencing this intention. We conducted face-to-face structured interviews with 1,039 parents at public health vaccination centres in four cities of the Campania region of southern Italy. Most respondents (91.9%) said that they would certainly (69.4%) or probably (22.5%) vaccinate their children if vaccinations were not mandatory. The belief that vaccinations are effective and safe was positively associated with willingness to vaccinate their children, whereas having heard that autism is a possible adverse reaction to vaccination was inversely associated with willingness to vaccinate. Nevertheless, in the context of the relatively low 2012* vaccination coverage rates in Campania (under the national standard of 95%), our results suggest that eliminating mandatory vaccinations is likely to lead to current coverage rates decreasing to unacceptably low levels, significantly below 90%.

Introduction

In European countries, childhood immunisation is provided in many different ways. There are large differences in whether vaccinations included in the national programmes are recommended or mandatory. At the end of 2010, among the 27 member countries of the European Union, 13 had no obligatory vaccinations, and 14 had at least one vaccination that was mandatory. For example, vaccination against polio is mandatory for children and adults in 12 countries, diphtheria and tetanus vaccination in 11 countries, and hepatitis B vaccination in 10 countries [1].

In Italy, diphtheria [2], polio [3], tetanus [4] and hepatitis B [5] vaccinations are mandatory. Vaccinations against measles-mumps-rubella (MMR), pertussis, *Haemophilus influenzae* type B, invasive pneumococcal disease, and *Neisseria meningitidis* group C are recommended by the current national vaccination schedule and are offered free of charge. In recent years, health authorities, as national policy, have urged vaccination centres to apply the above four vaccines in the form of a hexavalent vaccine that also includes pertussis and *Haemophilus influenzae* type B for infant immunisations. Therefore, the hexavalent vaccine is offered as an alternative to the four mandatory vaccines. So, from a practical point of view, pertussis and *Haemophilus influenzae* type B vaccines are given as if they are mandatory. Furthermore, in Italy there are significant legislative differences, not only between regions but also over time, due to the strong contrast between attitudes favourable and opposed to the mandatory vaccination. Therefore, there is now less distinction between mandatory and recommended vaccinations than in the past, and the concept of 'mandatory' has become ambiguous. For many years, children were not allowed to attend school if they were not vaccinated [6], but this regulation has been abolished [7], and in recent years, all regions have complied with this abolition. Administrative sanctions for failing to vaccinate a child are rarely applied. If a parent refuses to vaccinate their child, the parent will be called to an informative conversation at the local health authority in an attempt to gain compliance. Legislatively, each Italian region is relatively autonomous, so the laws regarding vaccination vary throughout the country. For example, in the Veneto region, vaccinations are not legally compulsory. After the suspension of obligatory vaccinations, this region has maintained high vaccination coverage. The Veneto region, making all vaccinations non-compulsory, appealed to the sense of responsibility of parents, who no longer had to feel 'forced' and could instead make decisions about vaccinations in a 'conscious' manner [8]. In considering a broader suspension of obligatory vaccinations, it is also important

to understand which variables influence parental decisions about whether to immunise their children [9–16].

The objectives of this study were (i) to investigate parental willingness to vaccinate their children if vaccinations were no longer mandatory and (ii) to evaluate the variables that influence this intention. We conducted the study in an area of southern Italy with sociocultural characteristics different from the Veneto region of northern Italy.

Methods

Participants and setting

In the Campania region of southern Italy, decisions about vaccine types, purchasing, and supply management are centralised at the local health authority and at the regional level. Childhood immunisations are provided at specialised public health vaccination centres (Unità Operative Materno-infantili).

We selected one vaccination centre from each of the four cities with the largest populations in the Campania region (Naples, Salerno, Caserta and Avellino). In Naples, we randomly selected one of the ten vaccination centres. Salerno, Caserta and Avellino each have only one centre. At each centre, two expert healthcare workers interviewed one parent of each child brought for the first, second or third dose of the hexavalent vaccine. When both parents were present at the centre, the workers interviewed only the mother. Written informed consent was obtained from each participant. Interviews were conducted immediately following the vaccinations on one or two days each week between January and April 2013, during all hours that the centres were open to the public.

Sample size

The target sample size of approximately 1,000 subjects was obtained by assuming a 95% prevalence of the main outcome, a precision of ±1.2%, a 95% confidence level and a power of 80%. A total of 1,039 questionnaires were completed (329 in Naples, 254 in Avellino, 251 in Salerno and 205 in Caserta). Using univariate analysis and chi-square tests, we analysed the parents' responses by demographic characteristics and location of vaccination centre. A value of $p<0.05$ was considered statistically significant.

Questionnaire

A questionnaire was developed, pilot tested in a full day of interviews in the Naples centre, and consequently modified. The aim was to investigate parents' vaccination intentions, the main outcome variable, by asking, 'Would you immunise your child if vaccinations were not mandatory?' Parents' intentions were scored on a five-point Likert scale, with values of 'certainly', 'probably', 'I don't know', 'probably not' and 'certainly not'. The 95% confidence interval (CI) for proportions was calculated for this outcome. In the analysis, the responses were dichotomised by distinguishing those answering 'certainly' and 'probably' from those giving any other response.

The parents were then invited to give their level of agreement with the following statements: 'I am favourable toward vaccination', 'Vaccinations are effective in reducing the risk of disease' and 'Vaccinations are safe' on a five-point Likert scale. The response categories were 'strongly agree', 'somewhat agree', 'I don't know', 'somewhat disagree' and 'strongly disagree'. For analysis, the responses were dichotomised to distinguish agreement ('strongly agree' and 'somewhat agree') from disagreement (all other response categories). Respondents were also asked to express their views on whether vaccinations should be mandatory or optional (response categories of 'entirely mandatory' and 'entirely optional') and on whether vaccinations can cause adverse reactions (response categories of 'never', 'rarely', 'frequently' and always were dichotomised as 'never'/'rarely' vs 'frequently'/'always').

The source of information on the possible adverse effects of vaccination was operationalised by asking, 'In which way have you been informed about the possible adverse reactions after vaccination?' Responses were dichotomised as physician vs other sources. Respondents were also asked whether any of their children had experienced adverse reactions after vaccination. Finally, two questions were asked concerning knowledge about the debate on autism as an adverse reaction and vaccines containing mercury. These questions were placed at the end of questionnaire to avoid influencing the responses to other items. First, respondents were asked, 'Have you ever heard of possible adverse reactions or diseases associated with the administration of vaccines containing mercury?' The second question was modified as a result of the pilot test. Initially, the question was, 'Have you ever heard of autism as a possible adverse reaction after the administration of vaccines?' As some parents were alarmed by this question, it was modified in the final questionnaire: 'Although this hypothesis was disproved, have you ever heard of autism as a possible adverse reaction after the administration of vaccines?'

Sociodemographic data were collected for each respondent: age, education level, marital status, occupation and number of children.

Statistical analysis

Bivariate tests were used to assess the univariate associations between each of the independent variables and the main outcome. Only those variables associated with the outcome at the $p\leq0.25$ level in the bivariate analysis were subsequently included in the multivariate regression model. Logistic regression models were then estimated to evaluate the association between the independent variables and the main outcome. Backward stepwise procedures were applied, and the final model included only variables contributing significantly to the explanation of the outcome. Variables

were selected for the multivariate model using p<0.2 for entry and p<0.4 for exclusion. Analyses were carried out using Stata 10 [17].

The final logistic model predicted parents' vaccination intentions 'Would you immunise your child if vaccination was not mandatory?' (no=0; yes=1). Based on the results of the univariate analysis, independent variables measuring attitudes towards vaccination and knowledge of potential adverse reactions following vaccination were included in the model. Specifically, the included independent variables were: being favourable toward vaccination (no=0, yes=1); believing that vaccinations are effective (no=0, yes=1); believing that vaccinations are safe (no=0, yes=1); believing that vaccinations should be mandatory (entirely optional=0, entirely mandatory=1); believing that vaccinations can cause adverse reactions (no=0, yes=1); source of information about possible adverse reactions after vaccination (physician=0, others=1); children's previous experience with adverse reactions after vaccination (no=0, yes=1); having heard of possible adverse reactions or diseases associated with the administration of vaccines containing mercury (no=0, yes=1) and having heard of autism as a possible adverse reaction after the administration of vaccines (no=0, yes=1).

Table 1

Selected characteristics of the study population, Italy, 2013 (n=1,039)

Characteristic	Mother		Father	
	n	%	n	%
Age (years)	927	100	109	100
15–19	12	1.3	0	0
20–29	229	24.7	15	13.8
30–39	516	55.7	50	45.9
40–49	165	17.8	39	35.8
50–59	5	0.5	5	4.6
Marital status	925	100	108	100
Married	807	87.2	101	93.5
Other	118	12.8	7	6.5
Education	927	100	109	100
Primary school	30	3.2	2	1.8
Middle school	215	23.2	18	16.5
High school	370	39.9	38	34.9
College degree	312	33.7	51	46.8
Occupation	926	100	108	100
Employed	465	50.2	100	92.6
Unemployed	461	49.8	8	7.4
Other children	926	100	109	100
Yes	492	53.1	60	55
No	434	46.9	49	45
Total[a]	**930**	**89.5**	**109**	**10.5**

[a] Numbers for each item may not sum to the total study population because of missing values.

Table 2

Parents' intentions to vaccinate their children, Italy, 2013 (n=1,039)

Would you immunise your child if vaccination were not mandatory?	n	%	95% CI
Certainly	721	69.4	66.6–72.2
Probably	234	22.5	20.0–25.1
Probably not	35	3.4	2.2–4.4
Certainly not	30	2.9	1.8–3.9
I don't know	19	1.8	1.0–2.6
Total	1,039	100	

CI: confidence interval for proportions.

The Ethics Committee of the Second University of Naples approved this study (reference number 41/2012).

Results

Sociodemographic characteristics

In total, 1,039 parents answered the questionnaire, and 34 (3.2%) refused to participate. Most of the respondents (89.5%) were mothers. Compared with the fathers interviewed, more of the mothers were between 20 and 39 years of age (80.4% of the mothers vs 59.6% of the fathers) and fewer were married (87.2% of the mothers vs 93.5% of the fathers). Respondents with more than one child made up 53.1% of the sample. Only half of the mothers were employed (50.2% compared with 92.6% of the fathers) (Table 1).

Intention to vaccinate

Among the 1,039 interviewed parents, 955 (91.9%) stated that they would certainly (n=721; 69.4%) or probably (n=234; 22.5%) vaccinate their children if vaccination were not mandatory. On the other hand, 84 parents (8.1%) stated that they would certainly (n=30; 2.9%) or probably not (n=35; 3.4%) vaccinate their children or that they did not know what they would do (n=19; 1.8%) (Table 2).

Attitudes and beliefs

Parents who intended to vaccinate their children if vaccination was not mandatory were generally more favourable toward vaccination and more likely to state that vaccinations are effective, safe (Table 3) and rarely or never cause adverse reactions (data not shown). Moreover, they thought that all vaccinations should be mandatory. In the bivariate analysis, all five included attitudinal variables were significantly associated with the main outcome (Table 4)

Knowledge about adverse reactions

Physicians were the main source of information about adverse effects, and intention to vaccinate was

Table 3

Parents' attitudes and beliefs about vaccinations, Italy, 2013 (n=1,039)

	I am favourable toward vaccination		Vaccinations are safe		Vaccinations are effective in reducing the risk of disease	
	n	%	n	%	n	%
Agree strongly	617	59.4	500	48.1	729	70.2
Agree somewhat	370	35.6	466	44.9	278	26.8
I don't know	12	1.1	15	1.4	11	1
Disagree somewhat	32	3.1	37	3.6	13	1.2
Disagree strongly	8	0.8	21	2	8	0.8
Total[a]	1,039	100	1,039	100	1,039	100

[a] Numbers for each item may not sum to the total study population because of missing values.

positively associated with this source of information. In the bivariate case, parents who had knowledge about potential adverse reactions of vaccines containing mercury or about autism as a consequence of vaccination reported the intention to vaccinate their children less often than those lacking this knowledge. Finally, having a child who had experienced an adverse reaction to vaccination did not affect the intention to vaccinate (Table 4). All data were disaggregated by centre location and by sociodemographic characteristics, but there were no statistically significant associations with the main outcome.

Multivariate analysis

In the multivariate analysis, the associations between the intention to vaccinate and the three variables on positive attitudes toward vaccinations remained statistically significant. These variables were: being favourable toward vaccination, believing that vaccinations are safe, and believing that vaccinations are effective. The association between not being favourable toward vaccination and knowledge about autism as an adverse effect after vaccination also remained significant (Table 5).

Discussion

The ongoing debate about obligatory vaccination involves two opposing considerations. On the one hand, the main arguments in favour of obligatory vaccination are that it maintains a high coverage rate. On the other hand, arguments against obligatory vaccination claim that it goes against the principles of self-determination and freedom of choice in health matters [18-24]. Moreover, an additional reason for not making vaccines mandatory is that, psychologically, it invites opposition, because individuals object to being told what to do.

Table 4

Factors associated with intention to vaccinate, Italy, 2013 (n=1039)

	Intention to vaccinate			
	Yes		No	
	n	%	n	%
Favourable toward vaccination				
Yes	933	94.5	54	5.5
No	22	42.3	30	57.7
	χ^2=181.3, df=1, p<0.001			
	RR=10.54, CI: 7.44–14.94			
Belief that vaccinations are effective in reducing the risk of disease				
Yes	941	93.5	66	6.5
No	14	43.8	18	56.2
	χ^2=103.07, df=1, p<0.001			
	RR=8.58, CI: 5.54–12.61			
Belief that vaccinations are safe				
Yes	913	94.5	53	5.5
No	42	57.5	31	42.5
	χ^2=124.89, df=1, p<0.001			
	RR=7.74, CI: 5.33–11.25			
Belief that vaccinations should be mandatory				
Entirely mandatory	378	96.9	12	3.1
Entirely optional	577	88.9	72	11.1
	χ^2=21.07, df=1, p<0.001			
	RR=3.61, CI: 1.98–6.56			
Belief that vaccinations can cause adverse reactions				
Yes	393	87.9	54	12.1
No	562	94.9	30	5.1
	χ^2=16.85, df=1, p<0.001			
	RR=0.42, CI: 0.27–0.64			
Source of information about possible adverse reactions after vaccination (n=802)[a]				
Physician	453	93.6	31	6.4
Others	277	87.1	41	12.9
	χ^2=9.9, df=1, p=0.002			
	RR=1.45, CI: 1.1–1.89			
Child has experienced adverse reactions after vaccination (n=913)[b]				
Yes	268	93.7	18	6.3
No	572	91.2	55	8.8
	χ^2=.64, df=1, p=0.2			
	RR=1.39, CI: 0.83–2.33			
Heard about adverse reactions associated with vaccines containing mercury (n=1.038)[c]				
Yes	130	84.4	24	15.6
No	824	93.2	60	6.8
	χ^2=13.64, df=1, p<0.001			
	RR=0.44, CI: 0.28–0.68			
Heard about autism as a possible adverse reaction to vaccinations				
Yes	199	83.3	40	16.7
No	756	94.5	44	5.5
	χ^2=31.3, df=1, p<0.001			
	RR=0.33, CI: 0.22–0.49			

CI: 95% confidence interval; df: degrees of freedom; RR: relative risk.

[a] A third response option was 'None'.

[b] A third response option was 'I don't know/I don't remember'.

[c] One answer was missing.

Table 5

Multivariate logistic regression analysis predicting the intention to vaccinate children if vaccination was not mandatory, Italy, 2013 (n=1,039)

Variable	OR	SE	95% CI	p value
Log likelihood: −149.78, χ^2=123.01 (df=7), p<0.0001				
Favourable toward vaccination	11.7	5.2	4.9–27.9	<0.001
Belief that vaccinations are effective in reducing the risk of disease	9.4	5.4	3.04–29.21	<0.001
Belief that vaccinations are safe	4.2	1.73	1.86–9.4	0.001
Heard about autism as a possible adverse reaction to vaccinations	0.47	0.15	0.25–0.88	0.02
Belief that vaccinations should be entirely mandatory	1.97	0.79	0.9–4.3	0.09
Informed about adverse reactions after vaccination by a physician (n=802)[a]	0.64	0.2	0.34–1.2	0.17
Belief that vaccinations can cause adverse reactions	0.67	0.22	0.35–1.3	0.24

CI: confidence interval; OR: odds ratio; SE: standard error.

[a] The option "None" has been excluded.

In the past, there was strong concern in Italy that making vaccination non-compulsory would result in lower coverage rates. However, the possibility of an optional vaccination scheme has recently gained popularity. The natural experiment undertaken in the Veneto region beginning in 2008 was both informative and reassuring, as the region's vaccination coverage has remained above the National Prevention and Vaccination Program standard of 95% after the suspension of obligatory vaccinations (Piano Nazionale Prevenzione Vaccinale 2012–2014) [25]. Based on this experience, the Italian health authorities have frequently declared their intention to make vaccinations non-compulsory nationwide. However, this intention has never been realised because of fear of reducing coverage rates. These fears are driven by the knowledge that the significant sociocultural and economic differences between Italian regions make it impossible to use the experience from one region to make an accurate prediction for others. Indeed, sociodemographic statistics indicate that the population in southern Italy is poorer, less industrialised, has lower employment, and is less educated than northern Italy. These characteristics, known as 'the southern question', exist where this work was carried out, and contribute to the contrast with the Veneto region in northern Italy. In the most recent Italian report on vaccination coverage rates from 2012* [26], the Campania region had the lowest rate (with the exception of the autonomous province of South Tyrol), whereas the Veneto region, even in absence of obligatory vaccination, had a rate that was higher than Campania's and just below the median value for all regions (Table 6).

The present study investigated parents' willingness to vaccinate their children even in the absence of compulsory vaccinations. We found that 91.9% of the respondents reported that they would certainly or probably vaccinate their children. At first glance, and considering the confidence interval, this result seems quite positive. However, if we consider that 6.3% of parents stated that they would certainly or probably not vaccinate their children and that the coverage rates in Campania in 2012 are under the national standard of 95%, it seems likely that the suspension of obligatory vaccination would result in the current coverage rates declining to significantly below 90%. This would represent an unacceptably low level of coverage. These considerations support the caution with which the Italian health authority is addressing this issue.

As expected, and as shown by previous research, the intention to vaccinate is significantly associated with the belief that vaccines are safe and effective [27–31]. However, negative attitudes toward vaccinations were not associated with a significant decrease in the intentions of parents to vaccinate their children. In terms of knowledge about potential adverse effects of vaccination, having heard of autism as a possible side effect after the administration of vaccines was most strongly negatively associated with intention to vaccinate [32]. The effect of this variable is more consistent than the effect of believing that mercury contained in vaccines causes damage [33]. The results were consistent across the four studied cities and for individuals with varying sociodemographic characteristics, potentially providing evidence of homogeneous attitudes toward vaccination throughout the region.

Our results should be interpreted carefully because the willingness to vaccinate children is not the same as the actual behaviour of bringing children for vaccinations. For example, a well-known critical point is that the interviewee may be influenced to respond according to the perceived wishes of the interviewer. Therefore, it is likely that, in the absence of obligatory vaccination, both those who intended to vaccinate their children and those who did not intend to do so might reconsider their positions. Another limitation of the study was the underestimation of the fathers' opinions (i.e. we interviewed only the mothers even when both parents were present). Taking the decision to vaccinate is a complex

Table 6

Vaccination coverage by region, Italy, 2012

	POL3	DTP3	DT-DTP3	EpB3
Piedmont	96.5	96.5	96.6	96.3
Valle d'Aosta	96.3	95.7	96.4	95.9
Lombardy	96.7	96.5	98.8	96.5
South Tyrol[a]	89.3	89.3	89.4	88.8
Trento[a]	95.4	95.1	95.3	95.0
Veneto	94.7	94.6	94.7	94.4
Friuli-Venezia Giulia	95.3	95.1	95.5	94.7
Liguria	96.8	96.7	96.8	96.8
Emilia-Romagna	96.3	96.0	96.5	96.1
Tuscany	95.3	95.1	96.6	95.2
Umbria	97.5	97.3	97.4	97.2
Marche	97.6	97.5	97.6	97.3
Lazio	98.9	98.8	98.9	99.9
Abruzzo	99.7	99.7	99.7	99.7
Molise	97.6	97.6	97.6	97.6
Campania	93.3	93.3	94.2	93.3
Puglia	96.5	96.5	96.5	96.5
Basilicata	99.8	99.8	99.8	99.8
Calabria	95.8	95.8	95.8	95.8
Sicily	95.7	95.7	95.7	93.3
Sardinia	93.3	93.3	93.3	96.0

Vaccination coverage is expressed in percentages. Numerator: number of subjects vaccinated within 24 months of age with complete cycle (three doses); denominator: number of subjects in the respective birth cohort.

DTP3: diphtheria, tetanus and pertussis (third dose); EpB3: hepatitis B (third dose); POL3: polio (third dose).

[a] South Tyrol and Trento are autonomous provinces.

Source: Ministero della Salute-Direzione Generale della Programmazione Sanitaria- Ufficio VI [26].

process that presumably involves a discussion between both parents, but this process was too difficult to detect with a short interview. Moreover, the interviews were conducted in a health centre immediately after the administration of the child's vaccination, and this setting probably encouraged a greater amenability to vaccinations. Our population was also older, more urban, more educated, and more likely to be employed than the average regional population because the sample units were the four vaccination centres in the cities with the largest populations in the Campania region. Finally, the sample excluded all parents who did not bring their children for vaccination. These individuals belong to two main groups: parents who have difficulty accessing health services (passive absentee) and parents who decide do not vaccinate their children (active absentee) [11]. Future research should explore the motivations of parents who do not vaccinate their children, especially as the vaccination coverage rates in the Campania region are among the lowest in Italy. Understanding which of these two groups is prevalent in the region (passive or active absentee) is crucial for designing appropriate interventions.

* Authors' correction

At the request of the authors, the date was corrected from 2011 to 2012 on 5 September 2014.

Conflict of interest

None declared.

Authors' contributions

FA: principal investigator, designed the study, wrote the article. CPP: responsible for organization, coordinator of data collection and data entry, contributed to statistical analysis and interpretation. SM and AJVA: data collection and data entry. GS: statistical analysis and interpretation.

References

1. Haverkate M, D'Ancona F, Giambi C, Johansen K, Lopalco PL, Cozza V, et al. on behalf of the VENICE project gatekeepers and contact points. Mandatory and recommended vaccination in the EU, Iceland and Norway: results of the VENICE 2010 survey on the ways of implementing national vaccination programmes. Euro Surveill. 2012;17(22):pii=20183.
2. Obbligatorietà della vaccinazione antidifterica. [Obligatoriness of diphtheria vaccination]. Legge 6 giugno 1939, n. 891. [Law of 6 Jun 1939, no 891]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 1 Jul 1939, no152. Italian. Available from: http://www.comilva.org/sites/default/files/R_Legge_6.6.1939_n.891.PDF
3. Obbligatorietà della vaccinazione antipoliomielitica [Obligatoriness of polio vaccination] Legge 4 febbraio 1966, n. 51. [Law of 4 Feb 1966, no 51]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 19 Feb 1966, no 44. Italian. Available from: http://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:legge:1966-02-04;51@originale
4. Obbligatorietà della vaccinazione antitetanica. [Obligatoriness of tetanus vaccination]. Legge 20 marzo 1968, n. 419. [Law of 20 Mar 1968, no 419]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 19 Apr 1968 no. 100. Italian. Available from: http://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:legge:1968-03-20;419
5. Obbligatorieta' della vaccinazione contro l'epatite virale B. [Obligatoriness of hepatitis B vaccination]. Legge 27 maggio 1991, n. 165. [Law of 27 May 1991, no 165]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 1 Jun 1991, no. 127. Italian. Available from: http://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:legge:1991-27-05;165!vig
6. Regolamento per l'applicazione del titolo III del decreto del Presidente della Repubblica 11 febbraio 1961, n. 264, relativo ai servizi di medicina scolastica. [Regulation for the implementation of Title III of the Decree of the President February 11, 1961, n. 264, relating to the services of scholastic medicine. D.P.R. 22 dicembre 1967, n. 1518. [Presidential decree of 22 Dec 1967, no 1518]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 6 Jun 1968, no 143. Italian. Available from: http://www.normattiva.it/uri-res/N2Ls?urn:nir:stato:decreto.presidente:1967;1518~art47!vig=
7. Regolamento recante modificazioni al DPR 1518/67 in materia di vaccinazioni obbligatorie. [Regulation containing amendments to Presidential Decree 1518/67 concerning mandatory vaccinations]. D.P.R. 26 gennaio 1999, n. 355. [Presidential decree of 26 Jan 1999, no 355]. Gazzetta Ufficiale della Repubblica Italiana [Official Journal of the Italian Republic]; 15 Oct 1999, no 243]. Italian. Available from: http://www.edscuola.it/archivio/norme/decreti/dpr51198.html
8. Report sull'attività vaccinale dell'anno 2011: coorte di nascita 2009 e monitoraggio della sospensione dell'obbligo vaccinale.

[Report on the vaccination activity of the year 2011: birth cohort 2009 and monitoring of the suspension of obligatory vaccination]. Venezia: Regione del Veneto; Jul 2012. Italian. Available from: http://www.pdconsiglioveneto.org/public/DGR/2012-DGR%201873_AllegatoA.pdf

9. Prislin R, Dyer JA, Blakely CH, Jonson Ch D. Immunization status and sociodemographic characteristics: the mediating role of beliefs, attitudes and perceived control. Am J Publ Health. 1998;88(12):1821-6. http://dx.doi.org/10.2105/AJPH.88.12.1821
10. Benin AL, Wisler-Scher DJ, Colson E, Shapiro ED, Holmboe ES. Qualitative analysis of mother's decision-making about vaccines for infants: the importance of trust. Pediatrics. 2006;117(5):1532-41. http://dx.doi.org/10.1542/peds.2005-1728
11. Samad L, Butler N, Peckham C, Bedford H, Millennium Cohort Study Child Health Group. Incomplete immunisation uptake in infancy: maternal reasons. Vaccine. 2006;24(47-48):6823-9. http://dx.doi.org/10.1016/j.vaccine.2006.06.039
12. Szilagyi PG, Rodewald LE. Missed opportunities for immunizations: a review of the evidence. J Public Health Manag Pract. 1996;2(1):18-25. http://dx.doi.org/10.1097/00124784-199600210-00005
13. Gust DA, Strine E, Maurice E, Smith P, Yusuf H, Wilkinson M, et al. Underimmunization among children: effects of vaccine safety concerns on immunization status. Pediatrics. 2004;114(1):e16-22. http://dx.doi.org/10.1542/peds.114.1.e16
14. Salmon DA, Moulton LH, Omer SB, de Hart MP, Stokeley S, Halsey NA. Factors associated with refusal of childhood vaccines among parents school-aged children. Arch Pediatr Adolesc Med. 2005;159(5):470-6. http://dx.doi.org/10.1001/archpedi.159.5.470
15. Guellin BG, Maibach EW, Marcuse EK. Do parents understand immunizations? A national telephone survey. Pediatrics. 2000;106(5):1097-102. http://dx.doi.org/10.1542/peds.106.5.1097
16. Raithatha N, Holland R, Gerrard S, Harvey I. A qualitative investigation of vaccine risk perception amongst parents who immunize their children: a matter of public health concern. J Public Health Med. 2003;25(2):161-4. http://dx.doi.org/10.1093/pubmed/fdg034
17. Stata Corporation. Stata Reference Manual. Release 10.1. College Station: Stata Press; 2007. Available from: http://www.stata.com/
18. Walkinshaw E. Mandatory vaccinations: The Canadian picture. CMAJ. 2011;183(16):E1165-6. http://dx.doi.org/10.1503/cmaj.109-3993 http://dx.doi.org/10.1503/cmaj.109-3992
19. Walkinshaw E Mandatory vaccinations: The international landscape CMAJ. 2011;183(16):E1167-8. http://dx.doi.org/10.1503/cmaj.109-3993
20. Walkinshaw E. Mandatory vaccinations: No middle ground. CMAJ. 2011;183(16):1830-1. http://dx.doi.org/10.1503/cmaj.109-3994
21. Sim F, Mackie P. Ethics and equity: choice or compulsion? Public Health. 2012;126(2):85-6. http://dx.doi.org/10.1016/j.puhe.2012.01.001
22. Offit PA. Should childhood vaccination be mandatory? Yes. BMJ. 2012;344:e2434. http://dx.doi.org/10.1136/bmj.e2434
23. Salisbury DM Should childhood vaccination be mandatory? No. BMJ. 2012;344:e2435. http://dx.doi.org/10.1136/bmj.e2435
24. Stefler D, Bhopal R. Lessons to be learnt from other countries about mandatory child vaccination. BMJ. 2012;344:e4036. http://dx.doi.org/10.1136/bmj.e4036
25. Piano nazionale prevenzione vaccinale 2012-2014. [National vaccination programme 2012-2014]. Rome: Ministero della Salute. [Accessed 16 Jan 2014]. Italian. Available from: http://www.salute.gov.it/imgs/c_17_pubblicazioni_1721_allegato.pdf.
26. Adempimento "mantenimento dell'erogazione dei LEA" attraverso gli indicatori della Griglia Lea. Metodologia e Risultati dell'anno 2012. Roma, Maggio 2014. [Compliance with the supply of LEA through the LEA indicators. Methods and Results of the year 2012]. Rome: Ministero della Salute-Direzione Generale della Programmazione Sanitaria-Ufficio VI; May 2014. Italian. Available from: http://www.salute.gov.it/imgs/c_17_pubblicazioni_2067_allegato.pdf
27. Gust D, Brown C, Sheedy K, Hibbs B, Weaver D, Nowak G. Immunization attitudes and beliefs among parents: beyond a dichotomous perspective. Am J Health Behav. 2005;29(1):81-92. http://dx.doi.org/10.5993/AJHB.29.1.7
28. Kennedy AM, Brown CJ, Gust DA. Vaccine beliefs of parents who oppose compulsory vaccination. Public Health Rep. 2005;120(3):252-8.
29. Smith PJ, Kennedy AM, Wooten K, Gust DA, Pickering LK. Association between health care providers' influence on parents who have concerns about vaccine safety and vaccination coverage. Pediatrics. 2006;118(5):e1287-92. http://dx.doi.org/10.1542/peds.2006-0923
30. Jones AM, Omer SB, Bednarczyk RA, Halsey NA, Moulton LH, Salmon DA. Parents' source of vaccine information and impact on vaccine attitudes, beliefs, and nonmedical exemptions. Adv Prev Med. 2012;2012:932741. http://dx.doi.org/10.1155/2012/932741
31. Colgrove J, Bayer R. Could it happen here? Vaccine risk controversies and the specter of derailment. Health Aff (Millwood). 2005;24(3):729-39. http://dx.doi.org/10.1377/hlthaff.24.3.729
32. Stehr-Green P, Tull P, Stellfeld M, Mortenson PB, Simpson D. Autism and thimerosal-containing vaccines: lack of consistent evidence for an association. Am J Prev Med. 2003;25(2):101-6. http://dx.doi.org/10.1016/S0749-3797(03)00113-2
33. Schultz ST. Does thimerosal or other mercury exposure increase the risk for autism? A review of current literature. Acta Neurobiol Exp (Wars). 2010;70(2):187-95.